COMMUNITY ECOLOGY AND GENETICS OF MACROINVERTEBRATES IN PERMANENT MACARONESIAN STREAMS

by

LUCY CLAIRE KELLY

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Abstract

Lucy Claire Kelly

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Extensive community-based sampling and single-species genetic analysis were used to study factors driving stream invertebrate community assembly on islands. Macroinvertebrates and physicochemistry were surveyed in forty-two streams on La Palma, La Gomera, Tenerife and Madeira (Macaronesia). Island faunal relationships and the role of the stream and catchment environment in determining community composition were investigated with multivariate analyses; assemblage nestedness and species richness, occupancy and abundance were also examined. The relationship between genetic differentiation and range size was tested using allozyme variation in selected species.

Island species pools differed in community composition and species richness (total, and endemic), broadly as predicted by theory of island biogeography. Stream and island species richness were correlated, showing unsaturated, possibly dispersal-limited, communities, and stream faunas were nested, evidence that assemblages were not random (*e.g.* only generalist/dispersive taxa occur at species-poor sites). Endemics occurred in more streams than non-endemics, suggesting greater habitat availability for the former, but similar niche width, endemic and non-endemics having similar local abundance. Species richness, community composition and the abundances of individual species were correlated with stream physicochemistry, itself reflecting geology, rainfall, altitudinal zonation of vegetation and the intensity of stream exploitation.

Allozyme variation was surveyed in *Mesophylax aspersus* (Trichoptera: Limnephilidae) and *Wormaldia tagananana* (Trichoptera: Philopotamidae), respectively having widespread and localised distributions. Population structure supported the hypothesis that range size is, at least partly, limited by poor dispersal ability in *W. tagananana*. Genetic variation in *Ancylus striatus* (Gastropoda: Ancylidae) was typical of polyploidy and self-fertilisation/parthenogenesis. Breeding system has consequences for a species' colonisation ability, and may partially explain the wide distribution of *A. striatus* within the islands.

Variation in community composition reflected patterns at a range of scales. Biogeography determined the island species pool, whilst local physicochemistry determined richness and community composition *within* islands. Species characteristics that affect their colonisation and extinction probabilities (*e.g.* habitat selection at the local- and mesoscales, dispersal patterns and breeding system), influence both the local and regional species pools.

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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A programme of advanced study was undertaken, which included completing the University of Plymouth Research Methods module, workshops on the use of statistical computer packages, and informal training in fieldwork methods, invertebrate identification and cellulose acetate gel electrophoresis.

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Oral presentation: Patch occupancy, abundance and endemism of stream fauna on oceanic islands.
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Four informal seminars were given to the Benthic Ecology Research Group, Plymouth, on the following subjects, March 1999-August 2000:
dispersal and environmental filters in community assembly;
population differentiation in two caddisflies with contrasting distributions;
population genetics of a self-fertilising, polyploid mollusc;
and occupancy and abundance compared in endemic and non-endemic species.

Signed L. Kelly

Date 5.11.01

Chapter 1

Introductory Overview

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1.1 Introduction

1.1.1 The importance of islands in ecological and evolutionary studies

Island studies have played a significant role in the historical development of many areas of ecological and evolutionary theory (*e.g.* Darwin, 1859; Wallace, 1880; MacArthur and Wilson, 1967; Diamond, 1975), and continue to do so (Grant, 1998c). Their attractions to biologists include depauperate communities, isolation and, often, replication of conditions making them 'natural experiments'. Islands are good locations for study of the evolutionary radiation of taxa (Schluter, 2000), as well as for investigating the diversity, composition and assembly of biotic communities (Sergel and Báez, 1990; Paulay, 1994; Brown and Lomolino, 2000a). This is because islands are depauperate for their size compared to continental areas of comparable climate, yet rich in species not found elsewhere (Whittaker, 1998). It has been questioned whether the insights gained from studying islands can be applied to continental situations (Vitousek *et al.*, 1995) but, whilst they may have unique ecosystems and evolutionary processes (*e.g.* Samways and Osborn, 1998), studies on islands will continue to provide insights into the ecological, biogeographical and evolutionary forces that shape species diversity (Brown and Lomolino, 2000b).

Volcanic archipelagos, such as the Hawaiian, Society and Canary Islands, are increasingly used as model systems for studying evolution. This is because of: (1) their isolation and the consequent insular nature of speciation; (2) their tremendous range of environmental diversity; and (3) the known geological history of island formation, which provides a chronological template for evolution (Carson, 1990; Hollocher, 1998; Emerson

et al., 2000). Studies of among-island differentiation have played a central role in developing biogeographical and evolutionary theories, for example in the testing of correlations between morphological and ecological variation (Brown and Pestano, 1998). A more recently developed focus of research is the elucidation of phylogenetic relationships, allowing the reconstruction of historical dispersal events in order to explain present-day distributions (*e.g.* Thorpe *et al.*, 1995; Brown and Pestano, 1998; Grant, 1998c).

Islands often have characteristic community composition. The biota tends to be depauperate, thus individual species may be found in high densities (Thorpe and Malhotra, 1998). It also tends to be disharmonic, with taxonomic groups missing or represented in proportions differing from those of continental communities, due to dispersal filters, for example (Whittaker, 1998). Relict taxa, such as the palaeoendemic species of the Macaronesian *laurisilva* (Section 1.2) are often present, and adaptive radiations frequently occur (Paulay, 1994; Grant, 1998c; Whittaker, 1998; Schluter, 2000). The best-known examples are the radiations within Darwin's finches (*Geospiza* (Fringillidae)) on Galapagos (Darwin, 1859; Grant and Grant, 1998), the Cichlidae of the African Great Lakes (*e.g.* Rüber *et al.*, 1998) and Hawaiian Drosophilidae (*e.g.* Carson and Templeton, 1984; Hollocher, 1998). Population bottlenecks, occurring particularly at the time of island colonisation, enhance the tendency of isolated populations to evolve rapidly and differentiate from their source populations, an effect observed within archipelagos as well as between islands and the continent (*e.g.* Berry, 1998).

There are some evolutionary trends particularly associated with islands (Grant, 1998a; Whittaker, 1998). In the fauna, these include gigantism, dwarfism and flightlessness, for example the flightless Carabidae of Madeira (Wollaston, 1865) and flightless birds of the

Pacific islands (Steadman, 1995). In island floras, aborescence, wind pollination and adaptations of seed morphology to reduce dispersal are common (Carlqvist, 1974; Givnish, 1998). A further evolutionary trend identified on islands is the taxon cycle, whereby an archipelago is colonised by a broadly adapted, generalist species, which then evolves into several locally adapted, specialist species. These are more prone to extinction and are eventually replaced by new colonists, leading to faunal turnover (Ricklefs and Cox, 1972). This is a special case of the general phenomenon of faunal turnover on islands, the result of a dynamic equilibrium between immigration and extinction (McArthur and Wilson, 1967; Law, 1999).

Patterns can also be identified in the process of community assembly on islands. Species richness has been predicted to very with island area, isolation and habitat diversity (MacArthur and Wilson, 1967; Whittaker, 1998), whilst both chance and dispersal ability influence the composition of the island species pool, particularly on small, isolated islands (Grant, 1998a). Island species pools are therefore non-random subsets of the continental source pool, and the selective nature of immigration, establishment and extinction often produces nestedness in island faunas (Patterson and Atmar, 1986; Brown and Lomolino, 2000a). The order of arrival of species at a site can be important, due to 'priority effects' (*e.g.* Clarke *et al.*, 1998; Law, 1999) and interspecific heterogeneity in competitive and dispersal ability produces heterogeneity patterns such as the 'checkerboard' observed for Caribbean frugivorous birds by Diamond (1975). Competitive release and vacant niche space often increase the niche widths of species in island communities, particularly on smaller islands where there is less scope for adaptive radiation (*e.g.* Roughgarden, 1995).

There is a sense of urgency in the study of island faunas (Grant, 1998c), as they are particularly vulnerable to extinction through mankind's activities, through habitat loss, and the introduction of predators and superior competitors, for example. Extinctions from prehistoric times (*e.g.* Steadman, 1995) to the present day (*e.g.* Quammen, 1996; Clarke *et al.*, 1998) have been documented on islands. The taxonomic distinctness of some island endemics gives them a special importance in conservation terms, in that their extinction would cause a greater loss of genetic and morphological diversity than the extinction of a species with close relatives (Whittaker, 1998). In addition, human activities are transforming continents into ever more fragmented habitat 'patchworks', thus the insights gained from the study of oceanic islands are important as they can be applied to these other island-like situations, for example in the 'Single Large or Several Small' debate around reserve design.

1.1.2 The study of stream communities

Unidirectional flow of water, with associated transport of matter, produces a longitudinal environmental gradient within streams (Giller and Malmqvist, 1998). The fauna show particular adaptations for living in this environment, both minimising (with mechanisms to maintain their position in the stream) and utilising (with feeding and predator avoidance mechanisms) the effects of flow (Allan, 1995). However, the in-stream environment is also strongly influenced by the catchment and landscape through which it flows. An exchange of inputs and outputs of water, detritus, nutrients and fauna occurs (Hornung and Reynolds, 1995); there also are physical effects of landscape topography on gradient and flow, of soils and geology on water chemistry and substratum composition, and, more locally, effects such as shading by riparian vegetation.

Much effort has been directed towards the search for patterns in stream community composition, a major landmark being the River Continuum Concept (RCC) (Vannote *et al.*, 1980), based on the premise that the observed structure of stream benthic communities is intimately and predictably related to the physical conditions of stream geomorphology (Minshall and Petersen, 1985). Community structure is expected to show predictable change on moving from headwaters to large rivers, for example varying with a general downstream trend from coarse to fine substrata (Hynes, 1970; Allan, 1995; Giller and Malmqvist, 1998). The RCC has proved to be simplistic (Giller and Malmqvist, 1998), however, as gradual downstream physical change is itself an idealisation (*e.g.* Statzner and Higler, 1985; Statzner and Borchardt, 1994). For example, site-specific factors may over-ride the longitudinal gradient (*e.g.* Bott *et al.*, 1985), and the nature of physicochemical gradients and biotas vary with biogeographic regions (*e.g.* Rundle *et al.*, 1993; Ormerod *et al.*, 1994; Winterbourn, 1995). The frequency and severity of variation in the stream flow regime are additional important physical factors in determining stream invertebrate communities (Lancaster and Hildrew, 1993; Grimm, 1994; Hildrew and Giller, 1994), with the productivity of stream reaches reflecting the flood disturbance regime (Giller and Malmqvist, 1998).

A second line of investigation has focussed on the role of biotic interactions (*e.g.* direct and interference/diffuse inter-specific competition, and predation) in determining community composition (McAuliffe, 1984; Peckarsky, 1984; Minshall and Petersen, 1985; Malmqvist *et al.*, 1992; Hildrew and Giller, 1994; Tokeshi, 1994; Malmqvist and Eriksson, 1995). Some of the complexity of biotic interactions has been encompassed in the study of food webs (*e.g.* Pimm, 1982; Hildrew, 1996; Yule, 1996). However, these lack predictive power and the question of whether the over-riding influence on community composition is

'top-down' (the action of predators) or 'bottom-up' (productivity limitation) is not yet fully explored in natural communities (Giller and Malmqvist, 1998). Hypotheses of single processes, such as competition or predation, being the fundamental determinant of community composition have proved to be too limited in scope to fully explain community composition (Hildrew *et al.*, 1984; Kohler, 1992; Ricklefs and Schluter, 1993b; Hugueny and Cornell, 2000).

More recently the above ideas have been combined and supplemented with information on larger scale influences on the fauna to produce a multi-scaled approach to understanding stream community composition (Chapter 3) (Giller *et al.*, 1994). Community assembly is viewed as the product of both regional and local influences (Ricklefs and Schluter, 1993b; Milner *et al.*, 2000; Rundle *et al.*, 2000) with species passing through environmental and dispersal filters (Belyea and Lancaster, 1999) (Figure 1.1). The regional, landscape and catchment scales therefore have a hierarchical influence on the stream community, as they do on its physical character (Giller and Malmqvist, 1998). In particular, the study of the influence of environmental variation, at a range of scales, from the microhabitat to the catchment (Hornung and Reynolds, 1995) and beyond, has been developed into the habitat templet approach (Southwood, 1977; Frissell *et al.*, 1986; Richards *et al.*, 1997; Townsend *et al.*, 1997a, b). This involves predicting and testing associations of species traits with axes of environmental variation (*e.g.* temporal dispersal frequency with habitat disturbance frequency). Poff (1997) developed a complementary niche-based approach, describing species in terms of their functional relationships to habitat selective forces (see also Usseglio-Polatera, 2000).

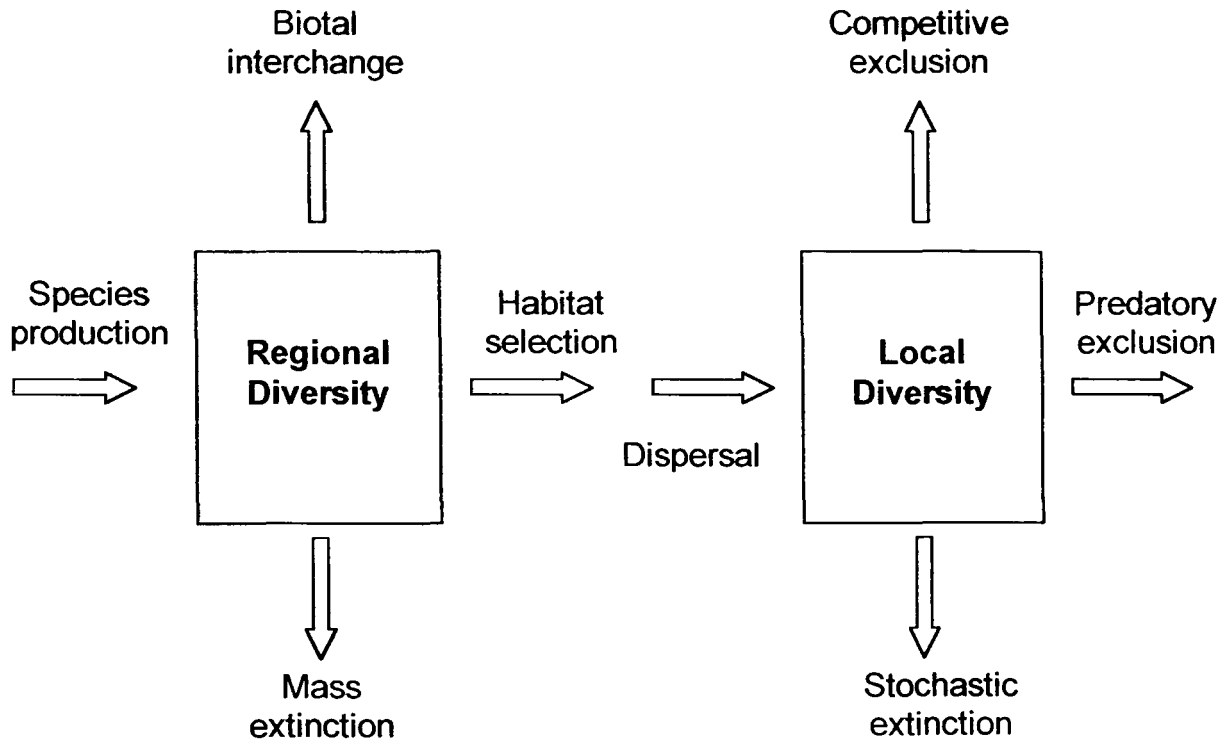


Figure 1.1 Influences on the regional and local species pools, and the relationship between regional and local species diversity. Figure adapted from Ricklefs and Schluter (1993a).

The species present in a stream are therefore those that are able to disperse to it (Section 1.1.3) and to tolerate the environmental conditions (*e.g.* flow, water chemistry, substratum, organic matter), including their temporal variation. For long-term persistence, individuals of a species must also avoid predation and succeed in competition for resources to the extent where the birth rate exceeds the death rate within the population. The temporal and spatial heterogeneity within streams allows for resource partitioning (Tokeshi, 1994; Giller and Malmqvist, 1998), facilitating species' co-existence. In temperate streams, there are also clear seasonal cycles of community structure and function (*e.g.* Furse *et al.*, 1984; Rundle *et al.*, 1998; Murphy and Giller, 2000). On a larger temporal scale, successional patterns can be detected in newly formed streams (*e.g.* Milner, 1994) and after disturbance events (*e.g.* Grimm, 1994), determined by species' ecological requirements, dispersal ability and the availability of a suitable source pool (Anderson and Wisseman, 1987). Local diversity, within streams, has also been shown to be dependent upon regional diversity (Chapter 3) (Vinson and Hawkins, 1998; Hugueny and Cornell, 2000) and biogeographic patterns, including speciation (Chapter 4) (*e.g.* Malmqvist *et al.*, 1995, 1997), the principal process linking the scales being dispersal (including 'passive sampling' of the species pool by sites) (Giller and Malmqvist, 1998).

Knowledge of the ecology of freshwater macroinvertebrates is valuable as they are ecologically and economically important as food for fish, as indicators of habitat quality, and as a major constituent of aquatic biodiversity (Allan and Flecker, 1993; Malmqvist and Hoffsten, 2000). In addition, they play an important role in energy and nutrient cycling (Graça, 1993). Investigations of their taxonomy, distributions, interactions, assemblage associations with environmental variables and factors determining community assembly are therefore useful (Malmqvist and Hoffsten, 2000). Stream biodiversity is threatened by many

of man's activities, including climate change, agricultural practices, industrial pollution, water abstraction, overexploitation, habitat loss and degradation, and the spread of exotic species (Allan and Flecker, 1993).

1.1.3 The study of dispersal between streams

Dispersal plays an important role in community composition (Tokeshi, 1994; Palmer *et al.*, 1996; Belyea and Lancaster, 1999), not only by the presence or absence of species but also through priority effects and predator-prey dynamics; the importance of the stochasticity of dispersal in determining community assembly has long been recognised (*e.g.* Talling, 1951). Many species may show dispersal-limited distributions (Pulliam, 2000), and dispersal can therefore be viewed on both the immediate timescale, as an ecological process (*e.g.* Grimm, 1994; Hall *et al.*, 1994), or on a larger scale, as an historical event leading to colonisation of a site or region (Brown and Lomolino, 2000b).

Stream invertebrates are generally considered to have high dispersal capabilities, especially given the wide geographic distributions of some species (Bunn and Hughes, 1997), and a wide range of passive and active dispersal mechanisms, both within and between water bodies, are utilised (Sheldon, 1984; Mackay, 1992; Bilton *et al.*, in press). Studies emphasising the importance of within-stream dispersal via drift in the water column dominated earlier literature on freshwater invertebrate dispersal (Hynes, 1970; Elliott, 1971; Müller, 1982; Minshall and Petersen, 1985). However, for aquatic insects, winged adults are the most likely principal dispersive stage involved in movement between sites (Schmidt *et al.*, 1995; Bunn and Hughes, 1997).

Whilst some direct observations of the dispersal of winged adults have been made (*e.g.* Griffith *et al.*, 1998; Petersen *et al.*, 1999), indirect study using genetic analysis of gene flow and population differentiation (Chapter 5) may give insight into dispersal patterns over larger spatial and temporal scales (Johnson and Black, 1995; Bilton *et al.*, in press). High dispersal rates between populations lead them to be genetically similar, whilst low dispersal rates, whether due to geographic barriers or poor dispersal ability, lead to population differentiation (Slatkin, 1985a). Therefore, the study of genetic differentiation can be used to infer the extent, and even potential mechanisms, of dispersal (*e.g.* Jackson and Resh, 1992; Schmidt *et al.*, 1995; Bunn and Hughes, 1997).

1.2 The Canary Islands and Madeira, Macaronesia

1.2.1 Location

The present study was carried out on the Canary Islands and Madeira, North Atlantic islands of the Macaronesian biogeographic province (Figure 1.2). This comprises the archipelagos of the Cape Verde Islands, Canary Islands, Madeira, Salvage Islands and the Azores. The Canary Islands are located in the Eastern Atlantic 200-500km from the coast of Morocco/Western Sahara, around 28°N 16°W. They form an archipelago of seven islands and four islets running east west; the westernmost of the Canary Islands studied, La Palma, lies at 28°N, 17°30'W and the easternmost, Tenerife, at 28°N, 16°40'W, with La Gomera between and slightly to the south. Madeira forms an archipelago with Porto Santo and the uninhabited Ilhas Desertas. Madeira lies at 33°N 17°W, 400km north of the Canaries and 620km from continental Africa. The area, age, degree of isolation and maximum elevation of the islands are listed in Table 1.1.

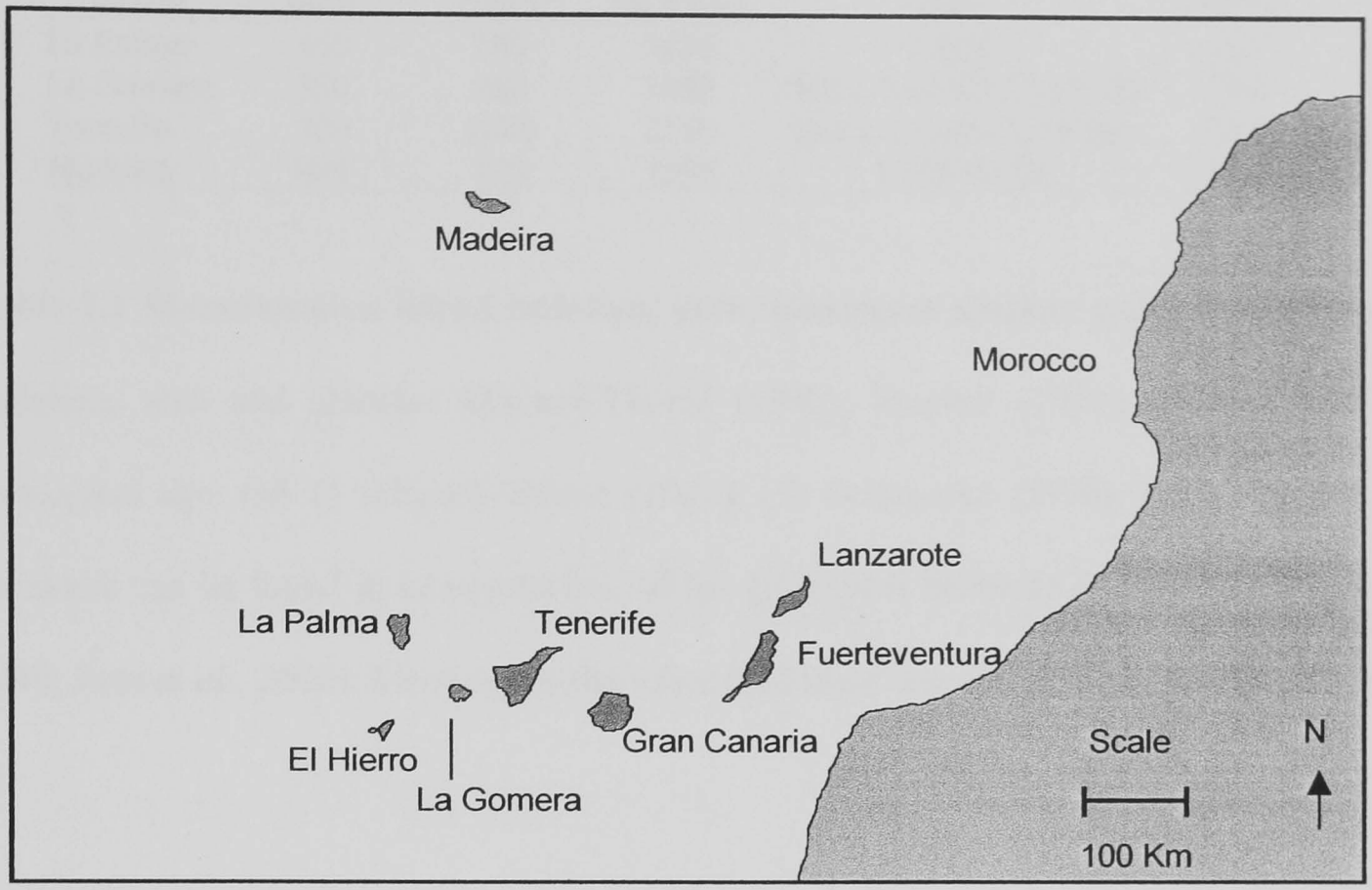


Figure 1.2 Location of the Canary Islands and Madeira, in the southeastern Atlantic.

The smaller islands of Porto Santo, Ilhas Desertas, Ilhas Selvagens and the islets of Lanzarote are not shown.

Island	Isolation (km)	Area (km^2)	Max. Alt. (m a.s.l.)	Age (MY)	Mean Age (MY)
La Palma	450	730	2426	2.0 (S)	2.0
La Gomera	380	380	1482	14.6 ± 0.67 (M-T), 13 (S)	14.6
Tenerife	300	2058	3718	15.9 ± 1.6 (M-T), 18 (S)	15.9
Madeira	620	720	1861	12-15 (M-T)	13.5

Table 1.1 Macaronesian island isolation, area, maximum altitude and geological age. Isolation, area and altitude: Mitchell-Thomé (1982); Stauder (1991); Hughes (1997). Geological age: (M-T) Mitchell-Thomé (1982); (S) Schmincke (1976). More recent age estimates can be found in interpretations of the geological literature (Brown and Pestano, 1998; Juan *et al.*, 2000). Mean age is the value used in subsequent analyses (Chapters 3 and 4).

1.2.2 Geology

The geological origin of the Canaries has been much debated but it has been concluded that all the islands, and also Madeira, are of volcanic origin and were not ever connected to each other or to the adjacent continent by land bridges (Rothe and Schmincke, 1968; Schmincke, 1976; Mitchell-Thomé, 1982). The islands are classified as an intra-plate cluster, the result of short, sub-parallel lines of crust weakness (Whittaker, 1998). The Canary Islands are in fact surrounded by deep water: the 100km shortest crossing to Africa is up to 1.5km deep, whilst the channels between the islands are over 2km deep.

The islands are of varying ages (Table 1.1), hence they have different physical characteristics and organisms have had different periods to colonise and evolve. On La Palma, Tenerife and Gran Canaria volcanic activity has been ongoing, so regions of the islands are of different ages; both Teide on Tenerife and Teneguia on La Palma have been volcanically active within historic times. Tenerife appears to have originated as three separate islands, recognised by older rocks in the areas of Teno, Anaga and Roque del Conde. These ancient islands became joined when the Cañadas and Teide volcanoes were formed, overlaying some areas with younger rock (Ancochea *et al.*, 1990, cited by Thorpe *et al.*, 1996; Juan *et al.*, 2000).

1.2.3 Climate

The Macaronesian islands have an oceanic climate with adiabatic rainfall, generated by cooling air forced to rise over higher land. The annual average temperature for the Canary Islands is 20-22°C, with a daily range of 5-7°C (Fernandopoullé, 1976). Temperatures are lower than usual for the latitude, due to the cooling effect of ocean currents and upwellings. Greater temperature variations are found at high altitudes, where

temperature inversions occur, and on south-facing slopes, which are often very sheltered as the prevailing wind is northeasterly. The climate in general is mediated by altitude, with snow being common in winter on the peak of Teide, Tenerife (Table 1.2). The climate of Madeira is similarly affected by damp northerly winds and, as there is a ridge running east west along the island, rain falls mostly on the northern aspect (2840mm per annum, compared to 600mm p.a. in the south) (Hughes, 1997).

1.2.4 Origins of the flora and fauna

The Canaries and Madeira have a fauna and flora of European, Mediterranean and African origin. The biota of Madeira is most similar to that of the Iberian peninsula, whereas a North African influence is a striking feature of the Canarian fauna and flora. Population affinities within species have also shown that Madeira has tended to be colonised from the North and the Canary Islands from Africa (Widmer *et al.*, 1998). There are additional elements in the biota with Southern hemisphere 'Gondwanan' distributions, for example some Dipteran genera (Báez, 1987), and others whose closest relatives occur in parts of Asia, for example *Hirudicryptus canariensis* (Diplopoda: Siphonocryptidae) and *Guanchia* (Dermaptera: Forficulidae) (Báez, 1987; Enghoff and Golovatch, 1995). Disjunct distributions such as these are the result of tectonic movement, chance dispersal events and, often, extinction in intervening areas, with the remote *laurisilva* of the Macaronesian islands acting as an evolutionary refuge (Enghoff and Golovatch, 1995). There are also taxa with disjunct distributions *within* Macaronesia; for example, *Cylindroiulus disjunctus* (Diplopoda: Juliidae) on El Hierro and La Palma is more closely related to *C. madeirae* on Madeira than to other Canarian species (Enghoff and Báez, 1993).

Altitude (m)	Temperature (°C)	Humidity (%)	Rainfall (mm p.a.)
0 - 250 North	20 - 22	75 - 85	200+
0 - 250 South	20 - 25	50 - 60	100+
250 -600 North	15 - 20	75 - 85	300 - 600
250 - 600 South	15 - 25	40 - 50	200 - 300
600 - 1000 North	15 - 18	60 - 70	500 - 800
600 - 1000 South	15 - 18	40 - 50	300 - 500
1500 - 2500 North	12 - 15	70 - 80	800 - 1000
1500 - 2500 South	12 - 15	50 - 60, fog	500 - 800
1500 - 2500 North	12 - 18 [a]	50 - 60, fog	800 - 1000, snow
> 2500	10+	50 +	800, snow

[a] Temperature inversion zone.

Table 1.2 The influence of altitude on temperature, humidity and rainfall on Tenerife. Temperature, humidity and rainfall ranges quoted are ranges of mean monthly values. Data are taken from Fernandopoullé (1976).

Many Macaronesian taxa show a high degree of endemism, attracting the attention of naturalists since the mid-Nineteenth Century. For example, endemism in the native flora of the Canary Islands is 27-45%, with the sub-alpine zone having over 90% endemism (Bramwell, 1990; Francisco-Ortega *et al.*, 2000). Around 50% of the terrestrial invertebrate fauna of the Canary Islands, and 27% of the total fauna of Madeira, is endemic (Báez, 1993; Juan *et al.*, 2000). The degree of endemism is enhanced by the islands' geographical situation, being ancient oceanic archipelagos yet, in the case of the Canary Islands, close to a continental species source. Having colonised, populations remain isolated for long periods of time, during which many have speciated (see Juan *et al.* (2000) for review). Isolated islands with naturally depauperate faunas also provide conditions conducive to adaptive radiation (Orr and Smith, 1998; Schluter, 2000); for example, Canarian floral species richness is enhanced by the radiations within *Aeonium* (Crassulaceae), *Echium* (Boraginaceae) and *Sonchus* (Asteraceae). Rapid adaptive radiation is generally facilitated on oceanic islands, where competition may be reduced and vacant niches are more numerous compared to continental biotas (Johnson *et al.*, 1984; Cameron and Cook, 1989). Among the freshwater macrofauna, *in situ* speciation may have produced the several endemic species of *Hydroptila* and *Stactobia* (Trichoptera: Hydroptilidae) (Schmid, 1959; Botosaneanu, 1981). A further important source of endemism is the presence of relictual species, which have become extinct elsewhere in their past range, and these include many of the trees and shrubs of the Macaronesian *laurisilva*, as well as a number of invertebrate taxa (Báez, 1987; Bramwell, 1990; Israelson, 1990; Enghoff and Golovatch, 1995).

1.2.5 Vegetation

Báez (1979) and Juan *et al.* (2000) provide simple classifications of the vegetation zones of the Canary Islands, which apply equally to Madeira, and which are a good

introduction to the islands' ecological communities. The islands are divided into three broad strata: (1) *piso basal*, the warm sunny coastal zone, up to 300m in altitude on northern aspects and 600m on south-facing slopes; (2) *piso montano*, the cool, humid cloud-level zone, up to 1500m; and (3) *piso subalpino*, the dry mountain-top zone (Table 1.2). This is above cloud level and experiences extreme variation in temperature by day and night. The low islands of Lanzarote, Fuerteventura, their islets and Madeira's neighbouring islands have only *piso basal* vegetation. All three vegetation types occur on La Palma, Tenerife, Gran Canaria and Madeira; *piso basal* and *piso montano* occur on La Gomera.

The *piso basal* vegetation is littoral and xerophytic. On Tenerife, irrigation enables the intensive production of bananas and tomatoes in the *piso basal* (Rodríguez Brito, 1995). The *piso montano* is extensively cultivated on most of the islands, with terracing on the slopes, where fruit, vegetables and vines are commonly grown. The natural vegetation is *laurisilva* between approximately 500m and 1000m altitude. This is evergreen woodland characterised by *Laurus azorica* (Lauraceae), *Ilex canariensis* (Aquifoliaceae) and *Persea indica* (Lauraceae) (Gandullo, 1991). Above the *laurisilva*, and intermingled with it on rocky ridges, is *fayal-brezal,* the scrubby woodland of *Myrica faya* (Myrtaceae) and *Erica arborea* (Ericaceae) up to 1500m. *Fayal-brezal* vegetation also occurs as secondary growth after felling or disturbance of the *laurisilva*. On La Palma and Tenerife, *Pinus canariensis* (Pinaceae) forest occurs at 1000-2000m on the drier slopes. At the highest altitudes, the vegetation of the *piso subalpino* is sparse and again xerophytic, dominated by *Parkinsonia aculeata* (Caesalpiniaceae) and *Echium* species.

1.2.6 Stream fauna

The permanent streams of the Canary Islands (on Gran Canaria, Tenerife, La Gomera and La Palma) and Madeira are spring-fed, with temporary streams flowing in the many *barrancos* (ravines) after heavy rain. The streams are mostly short, steep and first or second order, though there are some larger streams/rivers on Madeira. In the summer, flow may be reduced to a trickle between pools (Malmqvist *et al.*, 1995; Nilsson *et al.*, 1998).

The faunas of stony streams are remarkably similar the world over, with the exception of zoogeographically isolated islands (Hynes, 1970; Merritt and Cummins, 1984) and, typically, some components of the stream fauna are entirely absent from Macaronesia. For example, Plecoptera and several families of Coleoptera and Hemiptera are absent from the Canary Islands and Madeira, and freshwater Amphipoda are absent from Madeira. The non-endemic components of the Macaronesian stream fauna are predominantly European and Mediterranean on Madeira (Hughes *et al.*, 1998), with more North African species on the Canaries (Malmqvist *et al.*, 1995; Dobson, in press). The freshwater fauna of the Canary Islands is more species-rich than that of Madeira, which is more species-rich than that of the Azores.

Endemism is another feature of isolated islands that is evident in the Macaronesian stream fauna. There are high levels of endemism in many taxa (*e.g.* Coleoptera, Trichoptera and several families of Diptera) due to both *in situ* speciation and the presence of Tertiary relict species (Malmqvist *et al.*, 1995; Hughes *et al.*, 1998; Juan *et al.*, 2000). Madeira and Tenerife are particularly rich in endemic species (Table 1.3) (Malmqvist *et al.*, 1995; Hughes, 1997). Genetic exchange with continental source populations (where they exist) can be presumed to be a very rare occurrence (Stauder, 1991).

In contrast, some stream faunal groups show no endemism (*e.g.* Oligochaeta, Hirudinea and Mollusca on Madeira) (Table 1.3), which could be due either to more recent arrival on the islands, perhaps human-mediated, or to effective passive dispersal preventing genetic isolation (Stauder, 1991). There are also no endemics in the Canarian Ostracoda (Malmqvist *et al.*, 1997); their biogeography is similar to that of oceanic island ferns: both are predominantly parthenogenetic and have minute resistant propagules for dispersal, so have high colonisation and establishment abilities, given a suitable habitat. Whilst the level of endemism on the Canary Islands is generally high, there are relatively few single-island endemics within the freshwater fauna (Machado, 1987b; Malmqvist *et al.*, 1995), compared to the terrestrial fauna (Enghoff and Báez, 1993; Juan *et al.*, 2000). This suggests that inter-island dispersal is effective in much of the freshwater fauna (Boecklen, 1997; Kelly *et al.*, 2001).

1.3 Ecological and evolutionary studies on the Macaronesian islands

1.3.1 Introduction

Island biogeography is a recurring theme in the literature on the Macaronesian biota, both explicitly and implicitly, as the fauna and flora contain many examples of introduction and invasion, speciation, adaptation, relict species and peculiarities associated with the isolation of islands (*e.g.* Wollaston, 1864, 1865; Báez, 1987; Bramwell, 1990; Juan *et al.*, 2000). A survey of the more relevant literature is presented here; some papers are discussed in more detail elsewhere. The studies cited are mostly, but not restricted to, those on freshwater macroinvertebrates. Areas reviewed include major works on the taxonomy of Macaronesian stream invertebrates, and ecological and evolutionary studies of the island faunas.

Order/Group	Madeira			Tenerife		
Turbellaria	1			1		
Mollusca	10			5	(1)*	
Oligochaeta	8			19		
Hirudinea	2			2		
Hydracarina	25	(22)*	(2)**	6	(5)*	(3)***
Ostracoda	3			18		
Copepoda	1			?		
Isopoda	3	(1)*		0		
Amphipoda	0			1	(1)*	(1)***
Plecoptera	0			0		
Ephemeroptera	4	(1)*		8	(4)*	
Odonata	6		(1)**	11	(1)**	
Heteroptera	7	(1)*	(1)**	12	(2)*	
Coleoptera	21	(10)*		37	(12)*	(2)***
Trichoptera	15	(10)*	(3)**	14	(8)*	(2)** (2)***
Diptera [a]	44	(10)*	(13)**	47	(19)*	(5)** (5)***
Archipelago endemics	54			52		
Macaronesian end.	21			8		
Non-endemics [b]	55			121		
Total	130			181		

[a] Selected dipteran groups: Limoniidae, Psychodidae, Dixidae, Culicidae, Thaumaleidae, Simuliidae, Stratiomyidae, Empididae and Muscidae.

[b] Non-endemics includes species of unknown distribution.

Table 1.3 A comparison of species richness of freshwater invertebrates on Madeira and Tenerife. Numbers of species of selected taxa are shown (Báez, 1993; Malmqvist *et al.*, 1995, 1997; Hughes, *et al.*, 1998). Additional records are taken from Malicky (1999), Alarie and Bilton (in press), and the present study. * Endemic to archipelago, ** endemic to Macaronesia, *** endemic to Tenerife (included within number endemic to archipelago). For a similar analysis of various terrestrial groups see Báez (1987, 1992, 1993), Sergel and Báez (1990), Fernández-Palacios and Andersson (1993) and Borges and Brown (1999).

Speciation is a feature of many groups of organisms on the Canaries and Madeira, and the environmental conditions associated with different degrees of speciation and endemism are discussed in several studies. Finally, a number of genetic studies of the Canarian and Madeiran fauna and flora have been made, the majority investigating the phylogeny of speciating genera, relating the genetic relationships to the geographical distribution of species in order to make inferences about the sequence of island colonisation or population isolation.

1.3.2 Taxonomy

The aquatic macrofauna of the Canary Islands and Madeira has been quite thoroughly worked. Among the islands, the fauna of Tenerife is best known, followed by Gran Canaria and Madeira, whilst La Palma and La Gomera have received little attention from entomologists. Machado (1987a) compiled a bibliography of entomological publications referring to Canarian species (see Taxonomic Bibliography for key papers). In the earliest major works, Wollaston (1864, 1865) studied the Madeiran invertebrate fauna extensively, and visited other islands, describing many Coleoptera. McLachlan (1882) published on the Neuroptera and Odonata of the Canary Islands, primarily reviewing the work of Wollaston and Eaton. Nybom (1948, 1954) and others published findings of a Finnish expedition to the Canary Islands (1947-1951) that filled many of the gaps left by the Victorian naturalists. Knowledge of the taxonomy and distribution of some groups (particularly meiofauna) is still rather incomplete; recent descriptions include the endemic species *Simulium paraloutetense* (Diptera: Simuliidae) (Crosskey *et al.*, 1998) and *Polycentropus tenerifensis* (Trichoptera: Polycentropodidae) (Malicky, 1999). Taxonomic research has conservation importance in defining the status of endemic species (Malmqvist *et al.*, 1995).

1.3.3 Ecological studies

A number of ecological studies have been made on the freshwater and terrestrial invertebrate faunas of the Macaronesian islands. Spatial variation within a single stream was studied by Stauder (1991): invertebrate species were found to have different altitudinal ranges and substrate preferences, whilst, on a smaller scale still, the distribution within a stream of one functional guild, the surface dwelling predators Gyrinidae (Coleoptera) and Veliidae (Hemiptera), reflected inter-specific competition and predation (Malmqvist *et al.*, 1992).

Community composition of stream macroinvertebrates on Tenerife was investigated by Malmqvist *et al.* (1993, 1995). Within the island, streams differed markedly in their species composition, each having a unique assemblage of taxa and a different functional feeding guild composition. Species' ecological requirements also varied: more than 90% of taxa were pool or riffle specialists; some species showed a clear seasonal pattern in abundance whilst others did not. Armitage *et al.* (1996) found that lentic and lotic habitats on Tenerife had overlapping yet distinguishable chironomid faunas, but assemblages could not be related to environmental gradients such as altitude, perhaps reflecting opportunistic habitat use. In contrast, Ostracoda were associated with different water conductivities, altitudes and habitat types (Malmqvist *et al.*, 1997). The association of identifiable communities with physicochemical variables reflecting pollution gradients was used to develop a biological monitoring scheme for Madeira (Hughes, 1995), and Nilsson *et al.* (1998) analysed freshwater species richness and abundance on Gran Canaria in order to classify sites. Finally, the distribution of Trichoptera in the streams and canals of Madeira suggested differences between species in the relative importance of physicochemical and biotic factors in determining their presence or absence at a site (Hughes, 1997). While the

range of some species could be predicted by water temperature and chemistry, other distribution patterns appeared to reflect competitive exclusion and resource limitation. On a larger spatial scale, different terrestrial invertebrate communities were associated with distinct vegetation/climatic zones (Peraza *et al.*, 1986; Báez, 1979, 1988; Campos *et al.*, 1986).

Macroecological patterns have also been demonstrated in the freshwater fauna. Abiotic factors have been related to stream macroinvertebrate species richness: significant relationships were demonstrated with pool size, algal abundance, pH, altitude and temperature (Malmqvist *et al.*, 1993). Density, distribution and body size relationships of Gyrinidae and Veliidae in pools of different depths and areas were investigated by Malmqvist *et al.* (1992). Widely distributed species occurred at higher densities than those with distributions that are more restricted and, unusually, there was a positive relationship between mean body size and density. Among the Ostracoda, species with multi-island distributions occupied a significantly greater number of streams within islands. These widespread species are more generalist, or adapted to a more widely occurring habitat, than others (Malmqvist *et al.*, 1997). The general species-area relationship was found to hold within islands for dipteran species in *laurisilva* fragments (Báez, 1988).

1.3.4 Island biogeography

The variation between assemblages on different islands is an obvious pattern in the Macaronesian fauna (Machado, 1976), and the potential of the Macaronesian islands for testing theories of biogeography has been recognised (Quartau, 1982). Subjects investigated include island faunal relationships, the influence of island characteristics (*e.g.* area and isolation) on species richness, nestedness and faunal turnover modelled by the taxon cycle.

Borges (1990, 1992) suggested that the close relation of many Azorean endemics to Canarian and Madeiran endemics is evidence for stepping-stone colonisation, across the archipelagos; species similarity between islands generally decreases with distance (Fernández-Palacios and Andersson, 1993), but there are exceptions. For example, Canarian ostracod samples were found to be most similar to those from the Azores, whilst Madeira grouped with the Cape Verde Islands. Within the Canary Islands, samples from Tenerife, Gran Canaria and La Gomera grouped together, and those from La Palma and El Hierro formed a second cluster (Malmqvist *et al.*, 1997).

Island area, maximum altitude (representing ecological diversity (Whittaker, 1998)), geological age and isolation are often important predictors of species richness. This has been found to hold, in varying combinations, for many elements of the Macaronesian biota (terrestrial Coleoptera: Machado, 1976; avifauna: Báez, 1987, 1992; vascular plants, avifauna, Lepidoptera and Muscoidea (Diptera): Sergel and Báez, 1990; *Dolichoiulus* (Diplopoda: Juliidae): Enghoff and Báez, 1993; terrestrial Arthropoda: Borges and Brown, 1999). For example, in *Dolichoiulus* the greatest species richness occurs on the highest and largest islands, with few species on small, remote islands. There are also fewer on low, dry islands: the higher islands receive more rainfall and this contributes to the greater range of habitat. Finally, due to its younger age and isolation (see Table 1.1), the large high island of La Palma has fewer species than would otherwise be expected. These generalisations do not apply well to species with high dispersal capabilities, however, or those with very generalist requirements, or where human activities have determined present-day distributions (Báez, 1987, 1992; Sergel and Báez, 1990; Malmqvist *et al.*, 1997).

Aquatic Coleoptera and Ostracoda on the Canary Islands do not show the expected nested distribution patterns, that is the species present on species-poor islands are not subsets of those on species-rich islands (Malmqvist *et al.*, 1997). A coincidence analysis indicated that species in these groups have random rather than deterministic distributions, at the island scale. In terrestrial groups, attempts to find these patterns were equivocal. A hypothesis testing approach indicated random assembly of the avifauna (Fernández-Palacios and Andersson, 1993) in contrast to the conclusion reached by Báez (1992). For woody plants and Tenebrionidae, the null hypothesis of random colonisation was rejected and factors, such as distance to nearest land mass, prevailing wind direction and habitat availability, inferred, that is, deterministic patterns of faunal assembly (Fernández-Palacios and Andersson, 1993). Decreasing faunal similarity with increasing distance is consistent with non-random colonisation of islands, and the decrease is steepest for woody plants and Tenebrionidae, indicating low dispersal ability compared with the other groups investigated (land birds and butterflies).

Finally, the taxon cycle model was tested for Canary Island Hemiptera by Sergel and Báez (1990). Species density and number of habitats occupied were related to cycle stage, that is, the point species have reached in the evolutionary process from identity with mainland species to single-island endemicity. Báez (1992) envisaged a scenario of avifaunal turnover on the islands, that is, the relatively low number of endemic bird species and subspecies is due to an ongoing cycle of colonisation and extinction, with the possibility that the endemics are relicts of formerly more widespread species.

1.3.5 Patterns of endemism

The environmental conditions associated with different degrees of speciation and endemism are discussed in several studies. Reproductive isolation, genetic bottlenecks and habitat fragmentation caused by volcanic activity, may have provided the conditions necessary for rapid molecular evolution and the creation of new endemic species on the islands (Grant, 1998c), for example in *Calathus* (Coleoptera: Carabidae) on Tenerife (Machado, 1976; Emerson *et al.*, 1999) and in terrestrial Mollusca on Madeira (Cook *et al.*, 1990; Cook, 1996). Marginal isolation or niche specialisation of a generalist ancestor may have given rise to the abundance of *Calathus* species on La Gomera. Within genera invasion of new habitats appears to have been accompanied by a shift in resource use, for example there is some size differentiation of co-existing species of *Dolichoiulus* (Enghoff and Báez, 1993). *Laurisilva* has become a centre of biodiversity, particularly in terms of numbers of endemic species on the islands. There are four reasons for this: (1) as a habitat for relict fauna; (2) as a geographical refuge (from introduced species and from mankind's activities); (3) as a centre of speciation; and (4) as the location of the last stage of the taxon cycle, having many specialised species (Machado, 1976; Báez, 1988).

The species diversity and endemism in Coleoptera of the Azores, Madeira, Canaries and Cape Verde archipelagos were compared by Borges (1990). The Azores have relatively few endemic genera per family, explained by a poverty of biotopes due to constant climatic conditions, volcanic activity, destructive human activity, geographical isolation, young geological age, and insufficient search effort (Borges and Serrano, 1989; Israelson, 1990). Geological age was found to be equally or more important than island area in terms of the number of endemic species per island (Borges, 1990, 1992; Borges and Brown, 1999).

Several Canarian species show a remarkable degree of single island endemism. In the genus *Dolichoiulus*, possibly only one of the 46 species occurs on more than one island (Enghoff and Báez, 1993); *Cylindroiulus* on Madeira has radiated into 29 endemic species (Báez, 1993); *Calathus* has 24 species on the Canary Islands, none of which occur on more than one island (Emerson *et al.*, 1999); and *Brachyderes rugatus* (Coleoptera: Curculionidae) has a different subspecies on each of four Canary Islands it occupies (Emerson *et al.*, 2000).

1.3.6 Dispersal

Differing dispersal abilities of different groups of organisms have already been mentioned with regard to island biogeography (*e.g.* Fernández-Palacios and Andersson, 1993); however, some studies have focussed specifically on dispersal. Investigating the intra- and inter-island dispersal potential of macroinvertebrates, Ashmole and Ashmole (1988) sampled insects blown onto the snowfields of Teide or over the ocean, collecting a mixture of endemic, cosmopolitan and introduced species. On a larger temporal and spatial scale, Malacrida *et al.* (1998) used allozyme electrophoresis to trace the colonisation route of the medfly *Ceratitis capita* (Diptera: Tephritidae), from southeast Africa north to the Mediterranean and then southwest to the Canary Islands and Madeira. A study using a variety of genetic techniques has shown that Canarian populations of *Drosophila subobscura* have been isolated from mainland ones over several million years, whilst Madeira has been subject to continued immigration (Pinto *et al.*, 1997).

1.3.7 Phylogenetic studies

There has been much interest in the relationships between lineages on different islands in the Macaronesian fauna. Approaches towards constructing phylogenies have been

both morphological and genetic, using allozymes, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPD), ribosomal RNA and mitochondrial DNA) (Table 1.4).

The sequence of island colonisation within archipelagos may also be reconstructed, using a combination of phylogenetic and geological data (Thorpe *et al.*, 1995; Juan *et al.*, 2000). It has been found to reflect both island age, usually in the form of east to west stepping-stone colonisation (*e.g.* Gonzalez *et al.*, 1996; Juan *et al.*, 1997; Pinto *et al.*, 1997; Emerson *et al.*, 2000; Hess *et al.*, 2000), and more recent within-island volcanic activity (Brown, and Pestano, 1998). In particular, an effect of the three ancient islands of Tenerife, united by the eruption creating central Tenerife 1-2 million years ago, can be detected (Juan *et al.*, 1996a, b; Thorpe *et al.*, 1996; Emerson *et al.*, 1999).

Knowledge of organisms' phylogeny is valuable for the correct interpretation of morphological adaptations. Morphological traits of lineages, such as the skin colour of reptiles, have been related to the differing environmental conditions found on the islands, in particular variation in altitude, climate and vegetation (*Chalcides sexlineatus*: Brown and Thorpe, 1991; *Tarentola delalandii*: Thorpe, 1991; *Gallotia galloti*: Thorpe and Brown, 1991). These correlations are independent of geographical proximity and so provide evidence for selection acting upon the trait. Colour pattern variation did not reflect phylogeny, showing introgression between *G. galloti* lineages even after as much as 0.7 million years of separation (Thorpe *et al.*, 1996). Morphological and mitochondrial phylogenies of *Pimelia* were discordant, explained by rapid morphological change within each lineage as new habitats were exploited (Juan *et al.*, 1996a).

Morphological		
Thorpe *et al.*, 1985	*Gallotia* spp.	Squamata: Lacertidae
Enghoff and Báez, 1993	*Dolichoiulus* spp.	Diplopoda: Juliidae
Allozymes		
Borgen, 1996	*Lobelia canariensis*	Capparales: Brassicaceae
Pinto *et al.*, 1997	*Drosophila subobscura*	Diptera: Drosophilidae
Malacrida *et al.*, 1998	*Ceratitis capita*	Diptera: Tephritidae
Restriction Fragment Length Polymorphisms (RFLPs)		
Thorpe *et al.*, 1993	*Gallotia* spp.	Squamata: Lacertidae
Randomly Amplified Polymorphic DNA (RAPD)		
de Wolf *et al.*, 1998	*Littorina striata*	Gastropoda: Littorinidae
Ribosomal RNA		
Gonzalez *et al.*, 1996	*Gallotia* spp.	Squamata: Lacertidae
Mitochondrial DNA		
Juan *et al.*, 1996a, b, 1997	*Pimelia* and *Hegeter* spp.	Coleoptera: Tenebrionidae
Thorpe *et al.*, 1996	*Gallotia galloti*	Squamata: Lacertidae
Brown, R.P. and Pestano, 1998	*Chalcides* spp.	Squamata: Scincidae
Khadem *et al.*, 1998	*Drosophila subobscura*	Diptera: Drosophilidae
Nogales *et al.*, 1998	*Tarentola* spp.	Squamata: Gekkonidae
Widmer *et al.*, 1998	*Bombus terrestris*	Hymenoptera: Apidae
Emerson *et al.*, 1999	*Calathus* spp.	Coleoptera: Carabidae
Marshall and Baker, 1999	*Fringilla coelebs*	Passeriformes: Fringillidae
Emerson *et al.*, 2000	*Brachyderes rugatus*	Coleoptera: Curculionidae
Hess *et al.*, 2000	*Olea europaea*	Oleales: Oleaceae

Table 1.4 Phylogenetic studies on the Macaronesian flora and fauna. Studies are grouped by data type (not an exhaustive survey). The study organisms, their order and family are shown.

1.4 Conservation of running waters in Macaronesia

The varied landscapes of the Canary Islands and Madeira, and their unique fauna and flora, present special conservation problems. Having evolved in isolation, the biota is vulnerable to invasion by exotics (Whittaker, 1998), and the high degree of endemicity means that a large proportion of the species are globally rare: for example, over two-thirds of the endemic plant species are rare, threatened or endangered (Bramwell, 1990). An allozyme study of a wide range of Canarian endemic plants concluded that, whilst genetic diversity at the inter- and intra-population levels was high, efforts need to be made to ensure that it is conserved (Francisco-Ortega *et al.*, 2000). Space is at a premium on the islands and preservation of the natural heritage may be in conflict with the needs of the human populations and the expanding tourism industry. Areas of natural vegetation have been decimated over the last 500 years, particularly in the most recent decades (Gandullo, 1991). *Laurisilva* on Tenerife now covers only 10% of its natural extent, and on Gran Canaria only 1% (Bramwell, 1990). On Madeira, the reduction has been from 60% *laurisilva* cover to 16% in the 700 years that the island has been inhabited (Press and Short, 1994, cited by Wakeham-Dawson and Warren, 1998). *Laurisilva* is particularly sensitive as it does not regenerate well in the drier environment produced after fires or felling, being replaced by *fayal-brezal* vegetation (Gandullo, 1991).

The water quality and biodiversity of Macaronesian streams are increasingly threatened (Malmqvist *et al.*, 1993; Hughes, 1997). In addition, the very existence of natural running water on the islands is threatened, as the quantity of streams continues to be reduced. For example, on Gran Canaria the number of streams declined from 285 to 20 between 1933 and 1973, and to around eight semi-permanent streams by 1998 (Malmqvist

et al., 1993; Crosskey *et al.*, 1998; Nilsson *et al.*, 1998). Human activities threatening the biodiversity of running waters globally were reviewed by Allan and Flecker (1993); many of those activities are occurring on the Macaronesian islands (Malmqvist *et al.*, 1995). Pollution with sewage and agricultural chemicals lowers the quality of the running waters, whilst abstraction for intensive irrigated agriculture, the needs of the tourism industry and domestic use reduces the quantity, both directly and by lowering the water table (Rodríguez Brito, 1995). Abstraction methods utilised include capturing water into pipes, enclosed and open channels (*gallerias* and *levadas*), horizontal water mining and the drilling of bore holes. These result in streams becoming temporary in nature, with disconnected pools becoming stagnant. Deforestation, agriculture and development threaten stream margins and the catchment ecosystem as a whole. The destruction of natural vegetation also increases water run-off and soil erosion, as less water is retained on the vegetation and in the top-soil. In the past there would have operated a positive feedback effect of *laurisilva* forest cover, intercepting precipitation and cloud water and raising the water table, and stream flow (Gandullo, 1991; Wakeham-Dawson and Warren, 1998). Deforestation feeds back to increase aridity, resulting in the loss of *laurisilva* streams. The range sizes of specialist species may have reduced dramatically and the functional group composition of the stream fauna may have changed because of this habitat loss, for example replacing detritus shredders with algal grazers (Malmqvist *et al.*, 1993).

Several outstanding examples of the natural vegetation have National Park designation, but the water resources within these areas are not specifically protected. Vegetation restoration and environmental legislation may be beneficial in the conservation of some of the flora and fauna (*e.g.* Ibanez *et al.*, 1997; Wakeham-Dawson and Warren, 1998); however, preservation of water resources is essential for the conservation of other

elements. Studies of the aquatic communities and their biogeography are also very relevant to conservation of these organisms. First steps in the conservation of stream biodiversity are to identify what species are present, their habitat requirements and to assess which taxa are rare or threatened (Malmqvist *et al.*, 1995; Malmqvist and Hoffsten, 2000). Poorly dispersing species, those with restricted distributions and those with low genetic diversity are most vulnerable to extinction. Species-rich sites should be highlighted, as they are likely to be particularly valuable from the biodiversity perspective (Allan and Flecker, 1993). For example, a survey of permanent and temporary streams on Tenerife showed that temporary streams had on average only 50% of the species richness of permanent streams (Malmqvist *et al.*, 1995). The absence of a nested pattern has implications for site selection for conservation of aquatic taxa (Malmqvist *et al.*, 1997; Malmqvist and Hoffsten, 2000). If this is a general phenomenon then it is not possible to conserve all species, or even the majority, by preserving the most species-rich localities. The restricted or patchy distributions of many taxa mean that habitat loss is likely to accelerate extinction of certain taxa (Jeffries, 1989).

1.5 A study of stream invertebrate community structure and dispersal

1.5.1 The Macaronesian island streams as an ecological study system

As the studies reviewed above have illustrated, the Macaronesian islands provide an interesting opportunity to investigate several aspects of community composition. Whilst the stream faunas of Tenerife and Madeira have been studied before, to some extent (*e.g.* Malmqvist *et al.*, 1995; Hughes, 1997), the present study includes the first systematic survey of the freshwater invertebrate faunas of La Palma and La Gomera. It thus provides a unique opportunity for the analysis of faunal patterns across islands and habitats. This study will investigate the extent to which the communities are products of the isolated island

environment, and particularly the role of dispersal (inferred from population genetic differentiation) in producing the assemblages observed. Factors affecting the Macaronesian stream communities at several scales are investigated, from physicochemical characteristics of individual streams, to faunal relationships between islands and archipelagos.

The importance of patterns and processes in aquatic ecology operating at different scales was discussed by Giller *et al.* (1994), and the utility of a top-down, multi-scale approach to stream ecology was emphasised by Poff (1997) and Vinson and Hawkins (1998). In addition, many ecological concepts (*e.g.* species-area and occupancy-abundance relationships) have not been fully explored in the lotic environment: Fisher (1997) advocated the integration of current ideas in freshwater ecology with those the 'ecological mainstream'. Macroecological patterns (Gaston and Blackburn, 2000) such as the relationships between regional and local faunas (Figure 1.1) (Belyea and Lancaster, 1999; Lawton, 1999; Rundle *et al.*, 2000) and between species richness and environmental variables (Vinson and Hawkins, 1998; Malmqvist and Hoffsten, 2000) are beginning to be investigated. The opportunity is taken to examine the Macaronesian stream fauna for these macroecological patterns, relating them to the island situation of the study and comparing the patterns of endemic and non-endemic species.

1.5.2 Thesis structural overview

Chapter 2 describes the stream environment on La Palma, La Gomera, Tenerife and Madeira, using data collected on the physical and chemical variables that are likely to determine community composition by acting as species 'filters' at different scales (Wright *et al.*, 1984; Poff, 1997; Giller and Malmqvist, 1998). Significant physicochemical differences between streams on different islands and in different land use types were sought. The

physicochemical variation among the streams was then related to the invertebrate fauna in the following chapter, expanding upon the spatial extent of previous studies relating the island stream faunas to environmental variables (*e.g.* Malmqvist *et al.*, 1993; Hughes, 1997).

Chapter 3 is concerned with the macroinvertebrate communities, using abundance data from a quantitative sampling scheme, and presence/absence data collected with additional qualitative sampling. Hypotheses that species richness is determined by environmental variables, and by island biogeographical factors such as island age and isolation, were tested. The relationship between regional (island) and local (stream) species richness (Ricklefs, 1987; Caswell and Cohen, 1993; Lawton, 1999) was investigated, the first such analysis for the Macaronesian biota. The macroinvertebrate faunal assemblages were described, and analysed for differences between islands and land use types, and the influence of environmental variables tested (Statzner *et al.*, 1997; Townsend *et al.*, 1997a, b). This allowed the effect of local scale (stream characteristics), mesoscale (Holt, 1993) (different catchment land use types) and regional scale (inter-island) variation on species richness and community composition to be investigated.

A number of macroecological patterns (Maurer, 1999; Gaston and Blackburn, 2000) were investigated in Chapter 4, using species presence/absence data from the 42 study streams. Regional and evolutionary processes are expected to have a profound effect on the local communities, determining the species pool (Holt, 1993; Vinson and Hawkins, 1998). Firstly, a cladistic analysis (parsimony analysis of endemicity: Rosen, 1988) was used to illustrate the overall faunal relationships between islands. A nestedness analysis was performed, testing the extent to which the assemblages at species-poor sites are randomly

sub-sampled from the assemblages at more species-rich sites. Species richness of endemic and non-endemic taxa was compared, testing for significant differences between islands and land use types. Finally, occupancy (proportion of streams occupied) and mean local abundance of endemic and non-endemic species were calculated, enabling predictions about ecological differences between the two sets of species to be tested, and the occupancy-abundance relationship to be examined.

The second part of the thesis uses genetic differentiation, as revealed by allozyme electrophoresis, to investigate population genetic structure of selected species. Two caddisfly species, potentially active dispersers, with contrasting distributions, and a passively dispersing mollusc, were chosen. From gene flow, the relative dispersal ability of these species was inferred (Bohonak, 1999a). Thus, genetic studies were used to assess, indirectly, the role of dispersal in determining the observed species assemblages. To introduce this section of the thesis, Chapter 5 describes the use of electrophoresis to screen populations for allozyme variation and the interpretation of variation, making the first extensive review of the usefulness and contribution of allozyme studies to freshwater ecology. The chapter shows how allozyme analysis has been applied to topics ranging from taxonomy and phylogeny to dispersal, parasitism and reproductive systems. It is demonstrated that allozyme studies can be very informative about population structure and inter-population dispersal.

The first species selected for population genetic study was a widespread, non-endemic caddisfly *Mesophylax aspersus* Rambur, 1842 (Trichoptera: Limnephilidae), and the second a Canarian endemic caddisfly with a restricted distribution on the islands, *Wormaldia tagananana* (Enderlein, 1929) (Philopotamidae) (Chapters 6 and 7). Chapter 6

focuses on the electrophoretic method and interpretation of resulting population genetic data, whilst Chapter 7 tests the hypothesis that the species with the more limited distribution has lower dispersal ability, by comparing the population genetic structure of the two species.

Chapter 8 considers the population genetics of the Canarian endemic freshwater limpet *Ancylus striatus* Quoy and Gaimard, 1832 (Gastropoda: Ancylidae), and compares genetic variation and structure in this passively dispersed organism with that of the actively dispersing Trichoptera. The genetic data point towards this snail being polyploid (Städler *et al.*, 1993) and the possibility and consequences of a flexible breeding system, with both self-fertilisation and outcrossing occurring in populations, are discussed.

The final chapter brings together the different approaches taken to studying community assembly on the islands and discusses what conclusions may be drawn about the community composition and dispersal of the Macaronesian freshwater invertebrates. It emphasises the importance of scale, as well as heterogeneity of species properties. The observed species assemblages are the product of microhabitat scale biotic interactions and environmental conditions (niche availability), nested within island (and larger) scale species pool constraints (Belyea and Lancaster, 1999; Law, 1999). Such linking of regional and local scale processes (*e.g.* Ricklefs, 1987) is a relatively recent research direction in freshwater biology (Rundle *et al.*, 2000).

Chapter 2

Physicochemistry of Macaronesian Streams

Physicochemistry of Macaronesian Streams

SUMMARY

The physicochemical characteristics of 42 permanently flowing streams on four Macaronesian islands (La Palma, La Gomera, Tenerife and Madeira) were measured in order to assess differences in chemistry and physiography between islands and land uses. There were significant differences in conductivity, aluminium, altitude, water temperature, width and depth between islands (ANOVA, $p < 0.05$). In terms of water chemistry, streams on Tenerife had highest conductivity; those on La Gomera and Madeira highest aluminium concentrations. Streams on Madeira were also significantly wider, deeper, warmer and at lower altitudes than Canarian streams. Inter-island differences in water chemistry are likely to be related to differences in geological age, whereas differences in the physical nature of the streams on different islands are concordant with higher rainfall and lower exploitation of streams on Madeira. Streams in different land use types (*laurisilva*, pine forest and deforested land) did not differ in chemistry, but those in deforested land were narrower and at lower altitude than forest streams (ANOVA, $p < 0.05$). *Laurisilva* streams were, on average, cooler than others. The variation in stream physicochemistry with land use in part reflects the altitudinal zonation of vegetation on the islands. This study is the first to encompass all the permanent streams on the islands of La Palma, La Gomera and Tenerife, and provides environmental data for subsequent analysis in relation to the stream faunas.

2.1 Introduction

2.1.1 Stream physicochemistry and the biota

Stream chemistry varies naturally with geology, as well as with land use and contamination. This variation in chemistry often correlates with changes in invertebrate communities. For example, the stream faunas of mineral-rich and mineral-poor regions have been shown to differ (Wright *et al.*, 1984), and the faunas of acid streams are distinct from those of circum-neutral streams, with taxon richness normally showing a positive relationship with cation concentrations and pH (*e.g.* Rundle and Ormerod, 1991; Hornung and Reynolds, 1995; Vinson and Hawkins, 1998). Trace metals (*e.g.* aluminium, cadmium, iron, lead, zinc) can also significantly affect invertebrate communities (Gower *et al.*, 1994). Metal toxicity depends on numerous interacting biotic and abiotic factors (Campbell and Stokes, 1985; Havas, 1985; Gerhardt, 1993; Gower *et al.*, 1994).

The mechanisms by which water chemistry affects freshwater organisms may be both direct and indirect. Chemical conditions may be intolerable to some taxa, or may have sublethal effects, for example on reproductive success. Ion concentrations have a direct effect on freshwater organisms, as ion uptake is essential for homeostasis. In particular, continuous uptake of sodium, chloride, potassium and calcium is often necessary for survival (Sutcliffe and Hildrew, 1989). At extreme ion concentrations transport mechanisms for these ions become disrupted. The bioavailability of phosphorus and dissolved organic carbon are also affected by aluminium concentration (Vangenechten *et al.*, 1989). The strength of this effect depends on H^+ and calcium concentrations (Havas, 1985; Gower *et al.*, 1994). The concentrations at which trace elements become toxic or limiting are species-specific and, within species, there may be significant variation between populations (Mason, 1996).

Water chemistry may also affect invertebrate communities via several indirect mechanisms (Merritt and Cummins, 1984; Sutcliffe and Hildrew, 1989; Allan, 1995). These may act through bottom-up and top-down processes in the food web: the food supply to taxa may be altered due to an effect on primary productivity (a bottom-up effect) (*e.g.* Willoughby and Mappin, 1988), or the removal of predatory fish may alter predation pressure on invertebrate species (a top-down effect) and consequently alters inter- and intra-specific competition (Hildrew, 1996). Alternatively, the biota may be responding to some other factor, such as hydraulic regime, which co-varies with chemistry (Vinson and Hawkins, 1998).

The distribution of stream invertebrates at large (regional) to small (stream and microhabitat) scales is also influenced by numerous physical parameters (*e.g.* Poff, 1997; Townsend *et al.*, 1997b; Vinson and Hawkins, 1998). Such physical variables may describe the stream at the reach scale (*e.g.* depth, width, substratum composition) or the catchment/supra-catchment scales (*e.g.* land use, catchment area, geology). Flow rate, temperature and substratum composition are important determinants of invertebrate assemblage composition (*e.g.* Delucchi, 1988; Rundle and Ormerod, 1991; Hildrew and Giller, 1994), due to their role in structuring the environment at a scale perceived by the organisms. Flow, for example, can vary substantially between patches on the streambed, from areas of high forces to low flow refugia (Lancaster and Hildrew, 1993). Physical variables operate in a hierarchical manner, with catchment properties influencing reach-scale characteristics; altitude and land use, for example, can affect factors such as stream water temperature, flow velocity and channel morphology (Frissell *et al.*, 1986; Townsend *et al.*, 1997a; Giller and Malmqvist, 1998).

2.1.2 Stream physicochemistry on the Macaronesian islands

Some previous investigations have been made of the water chemistry of Tenerife and Madeira (Malmqvist *et al.*, 1993; Hughes, 1995, 1997). The present study included a more extensive survey of the streams on these islands, and the first survey of this kind on La Gomera and La Palma. The 31 streams surveyed on the Canary Islands represent almost all the permanent streams on these three islands. This study is therefore the first to be able to make an inter-island comparison of stream physicochemistry. Chemistry is expected to vary from island to island, as differing ages and geology produce different amounts of weathering (Giller and Malmqvist, 1998). Streams on older islands are predicted to be more mineral-rich than streams on younger islands. The physical nature of the streams is also predicted to vary with island, as island topography (potentially reflected in stream gradient and altitude) is related to geological age. Streams on the older islands of Tenerife and Madeira may, for example, be at lower gradients, and be more mineral-rich with higher conductivity, than those on younger islands, due to increased erosion.

Macaronesian streams flow through three types of land use: native evergreen laurel woodland (*laurisilva*) (Gandullo, 1991), native *Pinus canariensis* forest and deforested land (either fields or open areas close to villages or footpaths). Stream physicochemistry is expected to differ among land use types. Aluminium, phosphorus and pH have been shown to differ among streams flowing through coniferous forest, broad-leaved forest and agricultural land (Rutt *et al.*, 1989; Townsend *et al.*, 1997b) due to different capacities of the vegetation to retain nutrients and scavenge ions from the atmosphere (Hornung and Reynolds, 1995; Giller and Malmqvist, 1998). Ormerod *et al.* (1993) found that, for any given pH, aluminium concentrations were significantly higher in streams draining conifer than deciduous forest catchments. Some physical variables are also expected to vary as a direct consequence of land use, for example quantity of organic matter and shading

(Townsend *et al.*, 1997b; Giller and Malmqvist, 1998), whilst variables such as altitude and temperature may co-vary with land use (Rutt *et al.*, 1989).

The first aim of this chapter is to describe the physicochemical character of the Macaronesian streams, providing data for correlation with invertebrate assemblages (Chapter 3); water chemistry data are also examined for any concentrations of ions that could be high enough to influence invertebrate distributions significantly. Secondly, tests are made for significant differences in stream physicochemistry between islands and catchment land use types, to assess potential mesoscale patterns that might influence the stream biota.

2.2 Methods

2.2.1 Study area and sampling sites

The 42 study sites were first or second order streams on the Canary Islands and Madeira. The locations of the streams studied and the predominant catchment land use are given in Figures 2.1 (western Canary Islands) and 2.2 (Madeira), and Table 2.1. The survey strategy involved sampling *all* of the permanent streams on La Palma, La Gomera and Tenerife, plus a similar-sized, representative sample of permanent *laurisilva* streams (and one disturbed stream) on Madeira. A minority of the study sites were different reaches of the same stream, whilst others were tributaries within a catchment (Figure 2.3); however, for succinctness they are all treated as individual streams. Two typical streams in different land use types are illustrated in Figure 2.4. The streams were sampled on one occasion each, during a three week period in March-April 1998 (Canary Islands) and a five day period in June 1998 (Madeira). The close timings of sampling reduced seasonal effects on the inter-stream water chemistry differences explored.

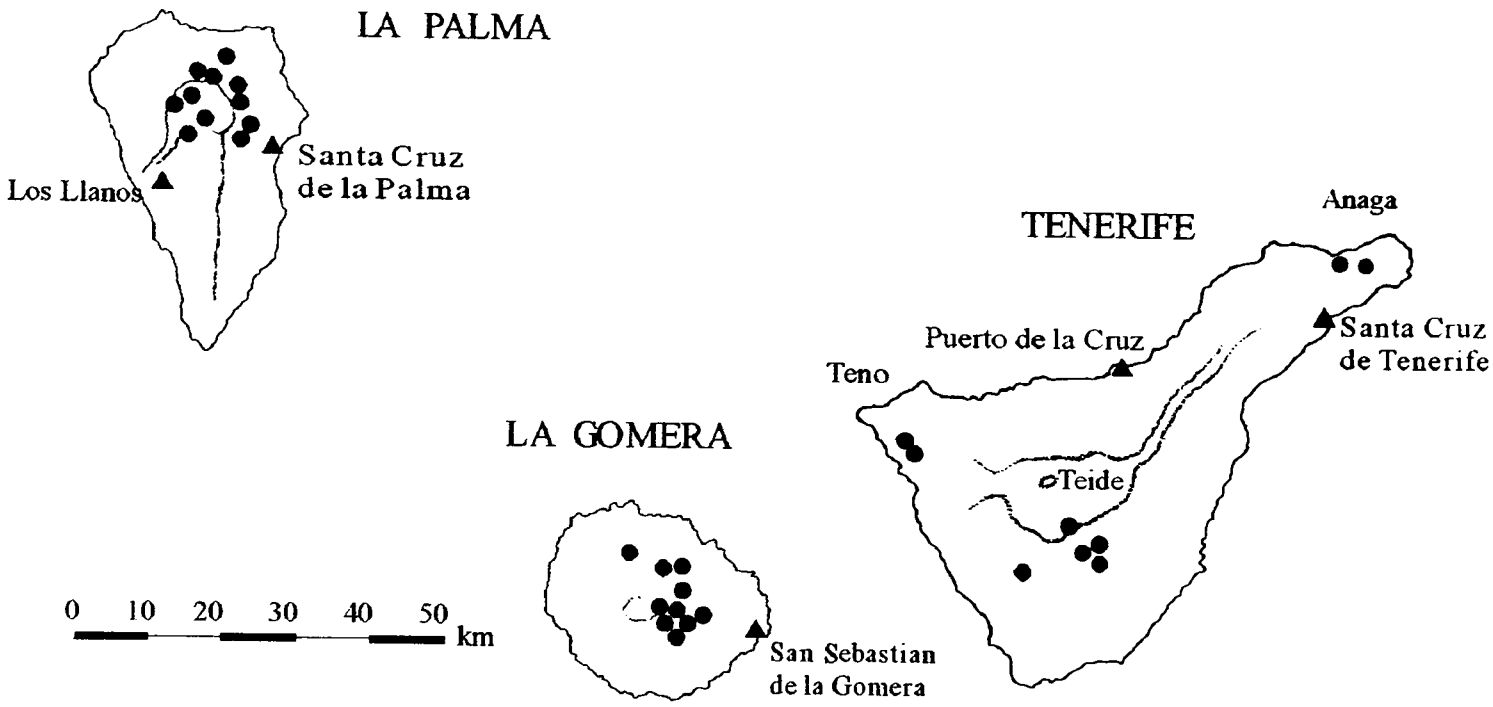


Figure 2.1 Locations of all permanently flowing streams on the western Canary Islands.

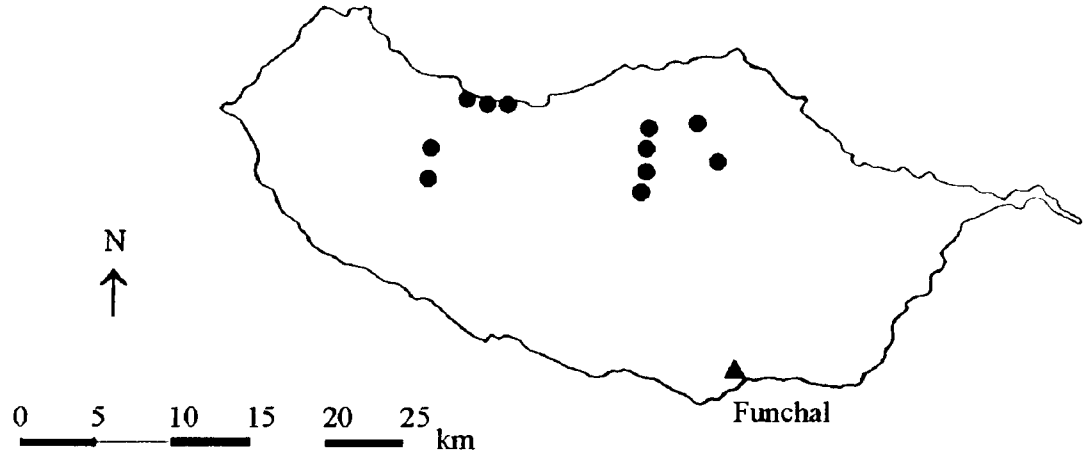


Figure 2.2 Locations of permanently flowing streams sampled on Madeira.

Site	Name	Grid. Ref.	Land Use
P1	Cubo del Galga (North fork)	284 844	Laurisilva
P2	Cubo del Galga (South fork)	286 846	Laurisilva
P3	Barranco del Agua, Los Tilos (low altitude pool)	254 865	Laurisilva
P4	Barranco del Agua, Los Tilos (spring above canal)	228 849	Pine
P5	Barranco del Agua, Los Tilos (high altitude pool)	235 853	Pine
P6	Rio Taburiente (channel closest to Zona de Acampada)	192 808	Pine
P7	Rio Taburiente (main channel)	192 806	Pine
P8	Barranco del Ciémpes, La Caldera	168 797	Pine
P9	Barranco del Tarves, La Caldera	172 803	Pine
P10	Barranco del Rio (main channel)	250 780	Laurisilva
P11	Barranco del Rio (right hand fork)	250 781	Laurisilva
P12	Barranco del Risco Liso, La Caldera	184 810	Pine
G1	El Cedro (first tributary upstream of car park)	817 124	Laurisilva
G2	El Cedro (second tributary upstream of car park)	817 123	Laurisilva
G3	El Cedro (third tributary upstream of car park)	816 123	Laurisilva
G4	El Cedro (main channel above G3)	816 123	Laurisilva
G5	El Cedro (main channel below G1)	818 124	Laurisilva
G6	El Cedro (beyond main channel)	821 144	Laurisilva
G7	El Cedro (before reaching main channel)	820 127	Laurisilva
G8	El Carmen, Valle Hermoso	771 159	Deforested
G9	Meriga	805 160	Laurisilva
G10	El Cedro (main channel below village)	825 138	Deforested
T1 [a, b]	Afur, North Anaga	778 592	Deforested
T2 [b]	Ijuana, Anaga	861 596	Laurisilva
T3 [b]	Masca, Teno	193 315	Deforested
T4	Masca (tributary), Teno	190 316	Deforested
T5 [b]	Barranco del Infierno	335 139	Pine
T6	Barranco del Rio (right hand tributary)	457 204	Pine
T7	Barranco del Rio (left hand tributary)	452 203	Pine
T8 [b]	Barranco del Rio (main channel)	459 193	Pine
T9	Barranco del Riocello, Las Canadas	404 214	Pine
M1	Risco	014 263	Laurisilva
M2	Ribeira dos Cedros	008 272	Laurisilva
M3	Ribeira da Sebastian Vaz	185 310	Laurisilva
M4	Rineiro de São Jorge	184 296	Laurisilva
M5	Levada das Faias, Quemada	165 299	Laurisilva
M6	Levada das Faias (fourth streambed/tributary)	159 296	Deforested
M7	Levada das Faias (tenth streambed/tributary)	157 287	Laurisilva
M8	Levada das Faias (eleventh streambed/tributary)	155 284	Laurisilva
M9	Seixal (westernmost stream of cluster)	037 324	Laurisilva
M10	Seixal (central stream of cluster)	038 324	Laurisilva
M11	Seixal (easternmost stream of cluster)	040 324	Laurisilva

[a] Malmqvist *et al.*, 1992
[b] Malmqvist *et al.*, 1993

Table 2.1 Location and catchment land use of 42 Macaronesian streams. Site codes: P: La Palma; G: La Gomera; T: Tenerife; and M: Madeira. Superscripts refer to papers in which more detailed site descriptions can be found. Names and grid references are taken from Cartografía Militar de España 1:50 000 and Carta Militar de Portugal 1:25 000 maps.

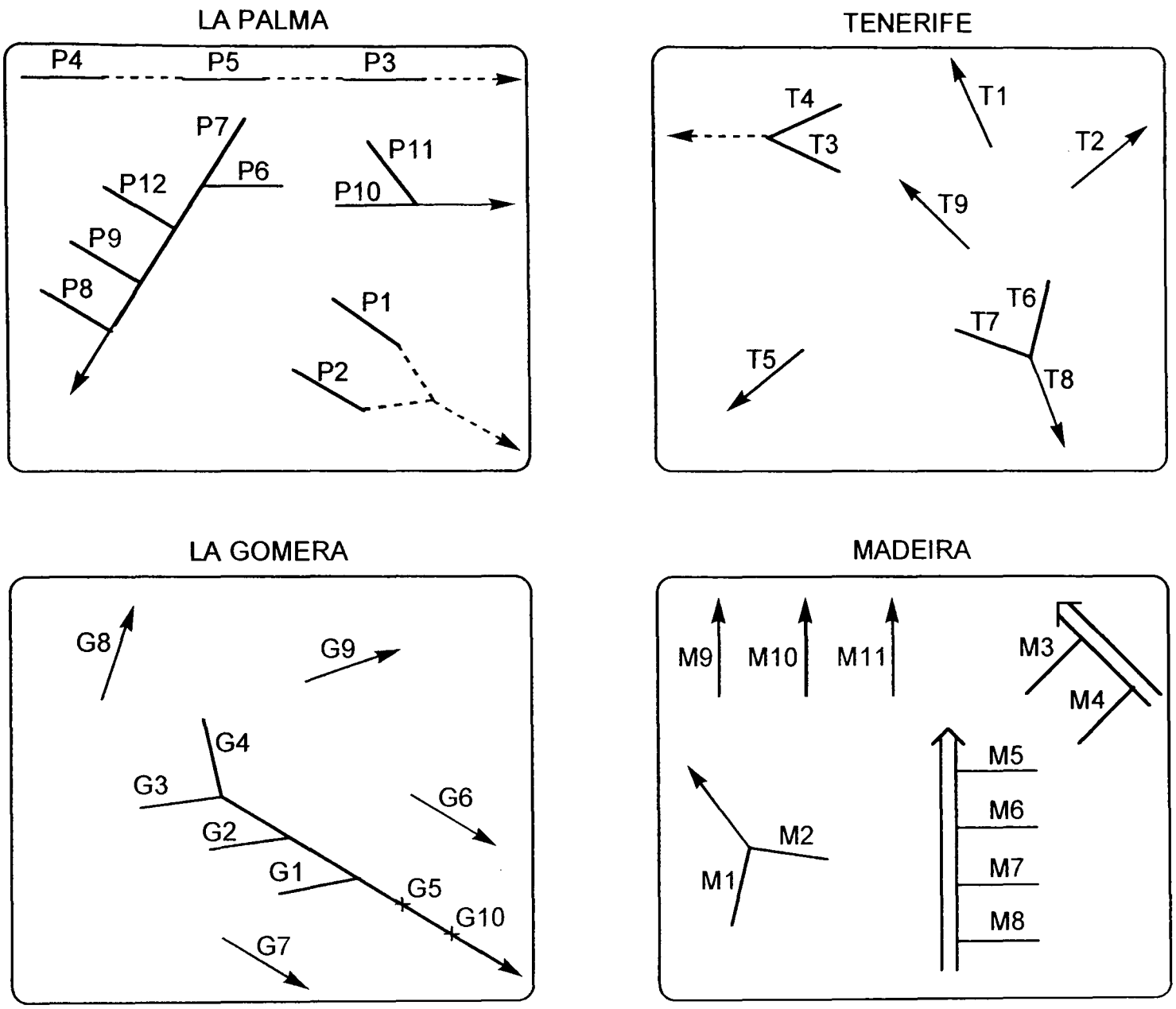


Figure 2.3 Diagrammatic representations of the geographical relationships between stream study sites on the Macaronesian islands. Solid lines indicate permanently flowing streams; dashed lines indicate dry streambeds/intermittently flowing streams; double lines indicate *levadas* (open water channels); and arrows indicate direction of stream flow. Not drawn to scale.

A

B

Figure 2.4 Two typical Macaronesian streams. A: site G4, El Cedro, La Gomera, a *laurisilva* catchment; B: site T4, Masca, Teno, Tenerife, a deforested catchment.

2.2.2 Water chemistry

The chemical variables quantified were those shown to influence stream biotas elsewhere (Section 2.1). Conductivity (in $\mu S\ cm^{-1}$) and pH were measured *in situ* with a Solomat 520c water quality meter, in an undisturbed pool in each stream. No pH readings were obtained for streams on Madeira, and so pH was excluded from multivariate analyses. Two 50ml water samples for metal and phosphate analysis were collected from the middle of a pool in each stream in early summer (March-June) 1998, using acid-washed polythene bottles. Samples were acidified with 2ml 5% nitric acid to fix metals, and stored at –20°C until analysis.

Trace metals were analysed in the laboratory with a VARIAN SPECTRA A-600 flame atomic absorption spectrophotometer. In this method, metal concentrations are determined by the amount of light from a cathode lamp absorbed by metal atoms as they change from the ground state under excitation by an energy source (in this case a flame). Concentrations of copper (Cu), zinc (Zn), aluminium (Al), iron (Fe), calcium (Ca) and magnesium (Mg) were determined. The lowest concentrations that could be detected at a 95% confidence level (the detection limit) are as follows: $2\mu g\ l^{-1}$ Cu; $1\mu g\ l^{-1}$ Zn; $18\mu g\ l^{-1}$ Al; $6\mu g\ l^{-1}$ Fe; $2\mu g\ l^{-1}$ Ca; and $0.2\mu g\ l^{-1}$ Mg. Calcium and magnesium concentrations were strongly correlated and so were combined into total water hardness, calculated as follows (Gower *et al.*, 1994):

$$\text{Hardness (mg } l^{-1}) = (Ca*2.50) + (Mg*4.12)$$

Soluble orthophosphate concentration was determined with a Technicon Autoanalyser II. Phosphates undergo a redox reaction with ammonium molybdate to form a blue phosphomolybdenum complex. The intensity of the colour change was measured with a colorimeter. The detection limit was $0.008mg\ l^{-1}$. In all the chemical analyses, readings of

zero were recorded as 0.001mg l^{-1} to represent concentrations below the detection limits of the analytical method.

2.2.3 Physical variables

Variables used to classify streams in terms of their physical nature were those used by Wright *et al.* (1984) for British streams and rivers: slope; altitude; depth; flow rate; variables relating to substratum composition; and macrophyte cover. Width (cm), depth (cm) and temperature (°C) were measured on site. Shade, flow rate and gradient were assigned a value from one (low/shallow) to three (high/steep). These approximate scales were used because of: the difficulty of quantifying shade as a point measurement; flow rate being too low, or stream size too small, to take a reading with a flow meter; and the gradient of the stream reach not being the same as the mean gradient over a larger scale as determined from a topographical map. Different substratum types were recorded as being dominant (5), abundant (4), frequent (3), occasional (2), rare (1) or absent (0). The substratum categories used were bedrock, boulders, cobbles, rocks, and gravel, sand and silt combined (Rutt *et al.*, 1989). Coarse and fine particulate organic matter (CPOM and FPOM) was recorded in the same way. Macrophytes were included with CPOM, and algae with FPOM. Altitude (metres above sea level) and distance (km) of the sampling site from the upper limit of the stream as marked on the map (a surrogate for distance from source) were determined from topographical maps.

2.2.4 Statistical analysis

The two metal and phosphate concentrations obtained were used to produce an average for each site. All chemical data other than pH were log transformed, as frequency histograms showed slightly right-skewed distributions. Draftsman's plots and calculation of Pearson's product-moment correlation coefficient were used to check for significant co-

linearity between variables. The data were then analysed using various programs in the PRIMER package (Plymouth Routines in Multivariate Ecological Research, Clarke, K.R. and Warwick, 1994).

To investigate the range of variation in physicochemistry among sites PCA (Principal Components Analysis: Clarke and Green, 1988) was used. Principal components are linear combinations of the variables with each component having as many terms as there are variables. The co-efficient of each variable in the linear combinations indicates its contribution to the component. A maximum of five principal components was specified. Each component explains a percentage of the variation in the data, with the first explaining the most. The second axis (perpendicular to the first) is the linear combination which best explains the remaining variation, thus the degree to which the two-dimensional ordination represents the data is given by the cumulative total variance explained by the first two axes. The variables were normalised and standardised by subtracting the overall mean value of the variable from each data point and dividing each by the standard deviation. The ordination was therefore scale-insensitive (essential when more than one unit of measurement is used in the set of variables) and the variances of the variables on the principal component axes were equalised, so the PCA is not influenced by inherently more variable measurements. This method produces a correlation-based PCA. Site G7 was excluded from the water chemistry PCA as inspection of the data revealed an anomalous, high concentration of iron at this site.

To test for differences in physicochemistry between islands and land use types, one-way analyses of variance were performed with ANOSIM (Analysis of Similarities: Clarke and Green, 1988; Clarke, 1993). Analyses were performed on the matrix of Euclidean distances, with significance determined by a permutation test (999

permutations). One-way analyses of variance on the raw data, with post-hoc multiple-range tests (least significant differences), were used to identify individual variables for which significant variation was explained by islands and land use.

2.3 Results

Several chemical and physical variables were significantly inter-correlated (Table 2.2). In further analyses calcium and magnesium were combined as hardness; all other variables were treated individually as the scatter of points about the regression lines was great (low R^2). (Raw data: Appendices 2.1 and 2.2).

The first water chemistry PCA axis (PC1) explained 22% of the variation among sites, and represented decreasing phosphate and iron concentrations, and increasing aluminium. PC2 brought the total variation explained to 40%, and represented decreasing copper and zinc, and increasing hardness (Table 2.3; Figure 2.5). PC1 for physical data explained 25% of the variation between sites and represented increasing width and boulders, and decreasing FPOM. The second axis explained an additional 15% of variation and represented increasing altitude, distance from source and depth, and decreasing temperature, cobbles and rocks (Table 2.4; Figure 2.6). The cumulative variation (40%) explained by the PCAs implied that the two-dimensional plots are not particularly complete representations of the data, but was high enough that broad trends in the data could be drawn out.

ANOSIM revealed significant differences between the four islands in terms of their water chemistry ($\rho < 0.002$, global $R = 0.135$, that is, 'island' explains 13.5% of the inter-site variation) and stream physical characteristics ($\rho < 0.001$, global $R = 0.158$). All island

	Cu	Zn	Mg	Ca	Al	Fe	PO4	Hard.	Cond.	pH	Alt	Temp.	Width	Depth
Zn	0.185													
Mg	-0.238	0.164												
Ca	-0.222	0.093	0.885 ***											
Al	-0.057	0.030	-0.023	-0.071										
Fe	-0.071	0.156	-0.051	-0.035	0.182									
PO4	-0.052	0.097	-0.034	0.045	-0.196	0.397 **								
Hard	-0.148	-.005	-0.120	-0.020	0.073	-0.032	0.216							
Cond	-0.215	0.125	0.879 ***	0.826 ***	-0.049	0.006	-0.009	-0.099						
pH	-0.093	-0.133	-0.091	-0.061	-0.512 ***	-0.162	0.025	0.143	-0.291					
Alt	0.207	0.052	-0.488 ***	-0.290	-0.096	0.164	0.178	-0.096	-0.248	0.078				
Temp	-0.318 *	-0.097	0.472 **	0.377 *	-0.213	-0.227	-0.060	0.293	0.348 *	0.189	-0.579 ***			
Width	-0.116	-0.047	0.160	0.300	-0.012	-0.197	0.142	-0.088	0.029	0.142	-0.091	0.106		
Depth	-0.082	0.182	0.164	0.147	-0.243	-0.162	-0.080	-0.253	0.153	0.153	-0.011	-0.015	0.414 **	
Source	-0.282	0.102	0.280	0.380 *	-0.088	-0.183	0.145	-0.143	0.318 *	-0.186	-0.088	0.109	0.514 ***	0.488 ***

Table 2.2 Correlation matrix for continuous physical and environmental variables from 42 Macaronesian streams. Pearson's product moment correlation co-efficients are tabulated, with significance indicated below (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	PC1	PC2
% Variation	20.5	19.1
Cum. % Var.	20.5	39.6
PO4	**-0.678**	0.168
Al	**0.511**	0.048
Fe	**-0.419**	-0.272
Hardness	-0.287	**0.450**
Zn	-0.122	**-0.391**
Cu	-0.071	**-0.718**
Conductivity	0.036	0.157

Table 2.3 Eigenvectors for PCA of water chemistry data for 42 Macaronesian streams. The table shows: percentage of inter-site variation explained by each axis; cumulative percentage of variation explained; and co-efficients of variables in the linear combination defining each axis. The highest co-efficients are highlighted in bold.

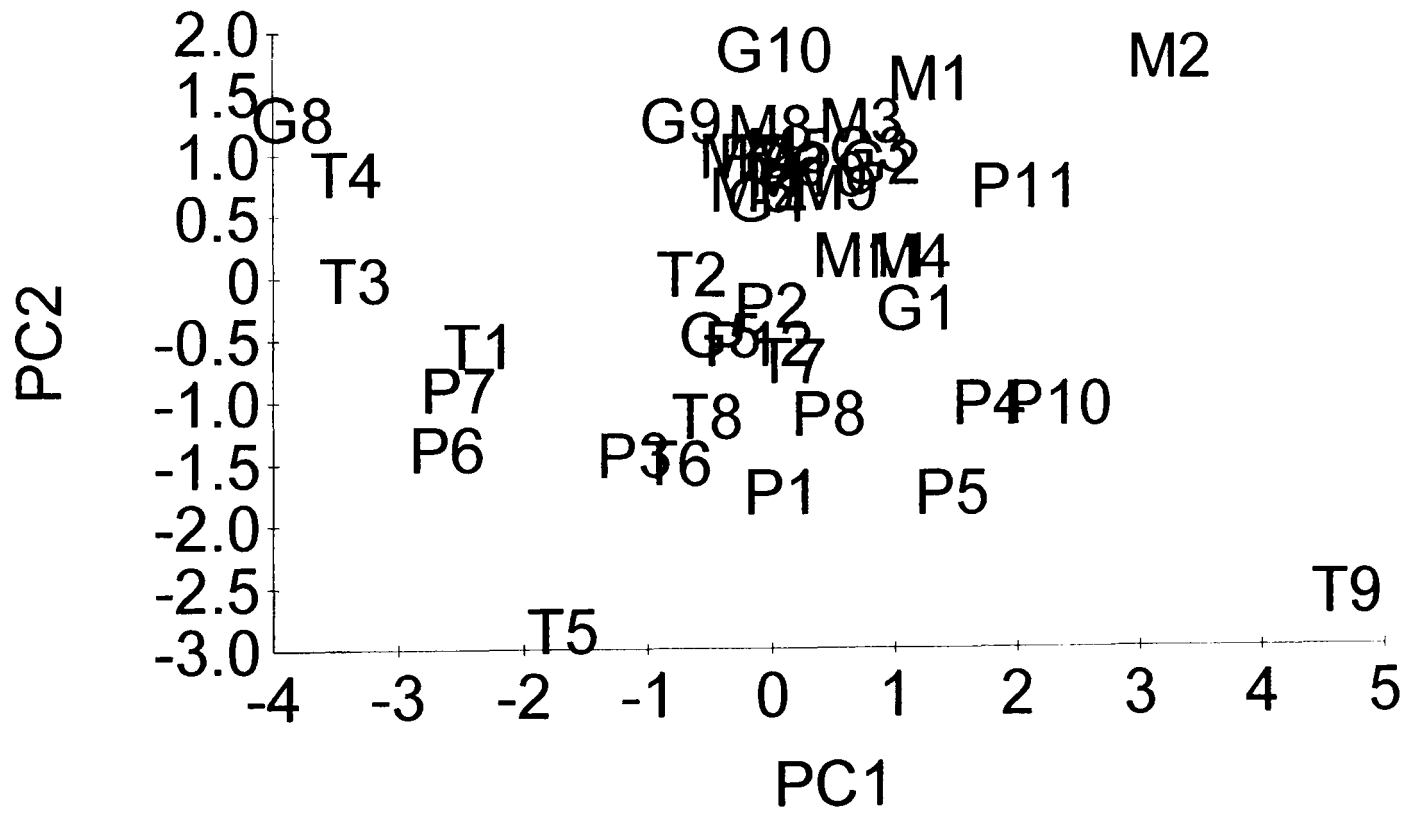


Figure 2.5 PCA of water chemistry data for 42 Macaronesian streams. See Table 2.1 for site codes. Site G7 is excluded.

	PC1	PC2
% Variation	25.3	15.2
Cum. % Var.	25.3	40.5
Width	**0.381**	0.245
Boulders	**0.353**	0.086
FPOM	**-0.321**	-0.013
Flow	**0.299**	0.273
Cobbles	**0.290**	**-0.313**
Gravel	-0.260	0.186
Temperature	0.255	**-0.322**
CPOM	-0.247	-0.103
Source	0.238	**0.314**
Depth	0.228	**0.348**
Rocks	0.215	**-0.335**
Altitude	-0.209	**0.419**
Shade	-0.196	0.069
Bedrock	-0.151	0.136
Gradient	0.006	-0.284

Table 2.4 Eigenvectors for PCA of stream physical data for 42 Macaronesian streams. The table shows: percentage of inter-site variation explained by each axis; cumulative percentage of variation explained; and co-efficients of variables in the linear combination defining each axis. The highest co-efficients are highlighted in bold.

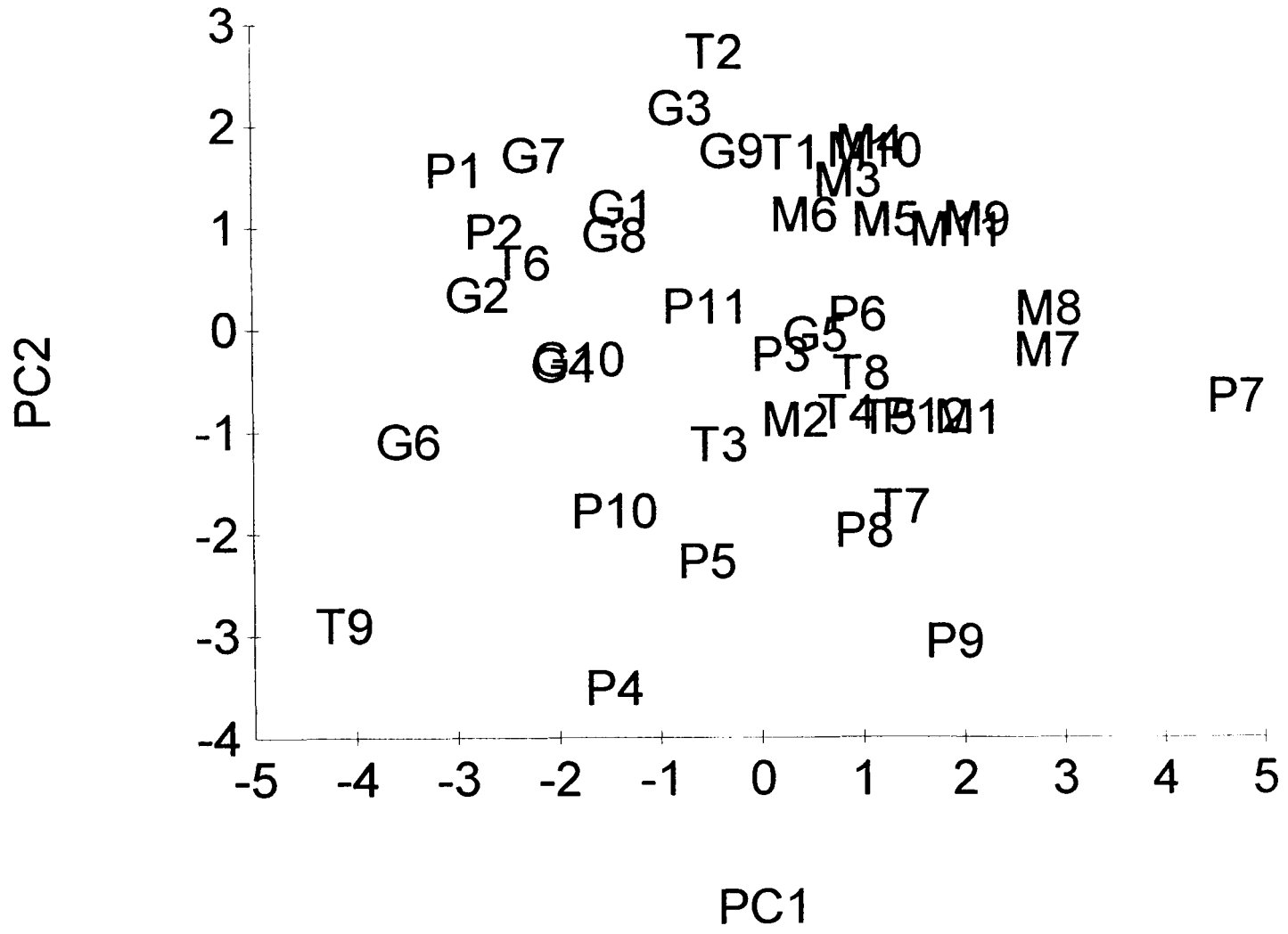


Figure 2.6 PCA of stream physical data for 42 Macaronesian streams. See Table 2.1 for site codes.

pairs except La Palma-Tenerife had significantly different water chemistry (ANOSIM, $\rho < 0.05$), though the average dissimilarity was low (< 12%). In addition, all island pairs except La Palma-Tenerife and Tenerife-Madeira had significantly different stream physical characteristics (ANOSIM, $\rho < 0.05$). There were significant differences between islands in terms of conductivity, aluminium, altitude, temperature and pH (ANOVA, $p < 0.05$, Table 2.5; Figure 2.7). On Figure 2.7, islands are labeled with letters (A, B, C): for each variable in turn, there is no significant difference between islands labeled with the same letter, but each differs significantly from those with which no letters are shared. Madeiran streams had significantly lower conductivity than La Gomera and Tenerife; La Palma also had significantly lower conductivity than Tenerife. Streams on La Palma and Tenerife had significantly lower aluminium than those on La Gomera and Madeira. Madeiran streams were at significantly lower altitude than Canarian streams and water temperature was significantly lower on La Gomera than on Tenerife and Madeira. Finally, pH was significantly lower on La Palma than on La Gomera and Tenerife.

ANOSIM demonstrated no significant difference overall between catchment land uses in terms of stream water chemistry or physical variables. However, significant differences in conductivity, aluminium, phosphate, altitude and temperature were found between land uses (ANOVA, $p < 0.05$, Table 2.6; Figure 2.6). Conductivities of streams in each land use type were significantly different, being highest in deforested catchments. Aluminium was significantly lower in pine forest streams than *laurisilva* streams. Pine forest streams also had significantly higher phosphate concentration and altitude than others. Finally, *laurisilva* streams had significantly lower temperature than others did.

	La Palma		La Gomera		Tenerife		Madeira		*F* ratio	*p* value
# Streams	12		10		9		11			
pH	7.85	*(0.42)*	6.60	*(0.188)*	6.74	*(0.10)*			61.27	0.001***
Cu ($\mu g\ l^{-1}$)	3	*(2)*	5	*(7)*	3	*(3)*	3	*(4)*	0.68	0.570
Zn ($\mu g\ l^{-1}$)	11	*(9)*	10	*(6)*	14	*(3)*	9	*(5)*	1.31	0.284
Al ($\mu g\ l^{-1}$)	35	*(75)*	1144	*(592)*	185	*(278)*	837	*(654)*	13.77	0.001***
Fe ($\mu g\ l^{-1}$)	139	*(85)*	394	*(742)*	163	*(134)*	68	*(33)*	1.50	0.229
PO_4 ($\mu g\ l^{-1}$)	111	*(33)*	85	*(59)*	130	*(100)*	83	*(15)*	1.48	0.235
Hard. ($mg\ l^{-1}$)	40	*(32)*	37	*(7)*	45	*(30)*	53	*(41)*	0.53	0.665
Cond. ($\mu S\ cm^{-1}$)	203	*(103)*	299	*(226)*	417	*(211)*	117	*(32)*	6.65	0.001***
Altitude (m)	968	*(347)*	914	*(148)*	973	*(695)*	471	*(297)*	3.85	0.017*
Source (km)	2.02	*(1.61)*	1.76	*(1.43)*	2.54	*(1.73)*	1.76	*(1.34)*	0.56	0.648
Temp. (°C)	13.5	*(1.2)*	11.9	*(1.0)*	14.5	*(3.0)*	14.9	*(1.6)*	4.84	0.006**
Width (cm)	126	*(168)*	87	*(53)*	114	*(89)*	154	*(97)*	0.62	0.604
Depth (cm)	20	*(11)*	16	*(5)*	18	*(10)*	13	*(7)*	1.39	0.260

Table 2.5 Mean physicochemical characteristics of streams on La Palma, La Gomera, Tenerife and Madeira. Continuous variables only analysed. Standard deviation is shown in italics. *F* ratios (41 degrees of freedom) and *p* values for one-way ANOVA are given. Significance is indicated: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

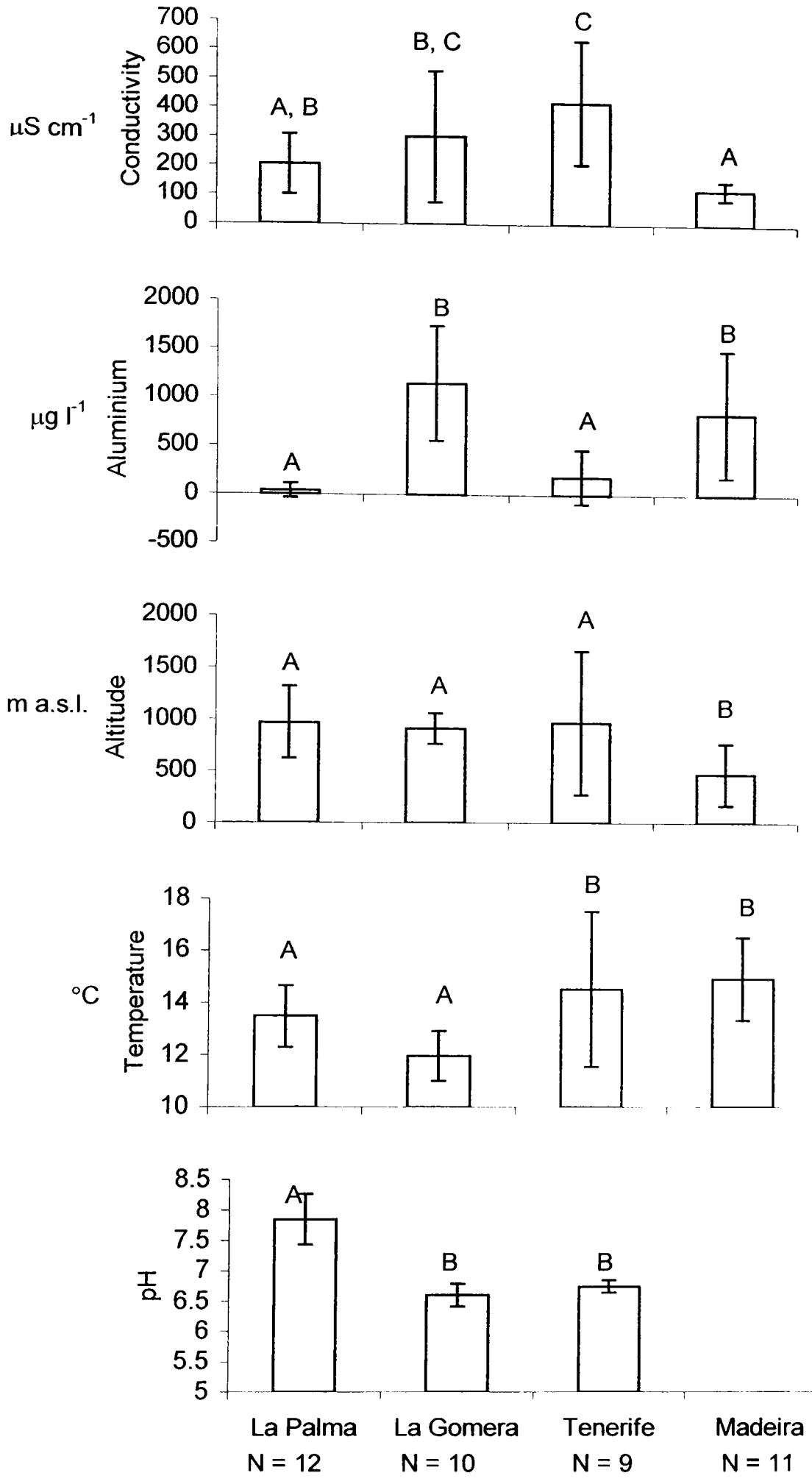


Figure 2.7 Physicochemical variations between streams on four Macaronesian islands. Letters indicate sets of islands within which differences are not significant (ANOVA, $p > 0.05$) (see text).

	Laurisilva		Pine		Deforested		*F* ratio	*p* value
# Streams	26		10		6			
pH	7.05	*(0.59)*	7.43	*(0.81)*	6.75	*(0.1)*	2.13	0.138
Cu ($\mu g\ l^{-1}$)	408	*(5)*	265	*(3)*	242	*(92)*	0.58	0.567
Zn ($\mu g\ l^{-1}$)	10	*(7)*	12	*(4)*	11	*(5)*	0.11	0.896
Al ($\mu g\ l^{-1}$)	736	*(709)*	26	*(78)*	556	*(370)*	5.35	0.009**
Fe ($\mu g\ l^{-1}$)	218	*(472)*	141	*(118)*	126	*(89)*	0.23	0.794
PO_4 ($\mu g\ l^{-1}$)	94	*(40)*	144	*(88)*	65	*(30)*	4.62	0.016*
Hard. ($mg\ l^{-1}$)	40	*(25)*	54	*(41)*	43	*(32)*	0.81	0.451
Cond. ($\mu S\ cm^{-1}$)	168	*(70)*	279	*(119)*	552	*(302)*	20.13	0.001***
Altitude (m)	784	*(387)*	1157	*(488)*	458	*(257)*	6.13	0.005**
Source (km)	1.73	*(1.33)*	2.44	*(1.97)*	2.55	*(1.3)*	1.13	0.334
Temp. (°C)	13.0	*(1.9)*	14.4	*(2.5)*	15.4	*(1.9)*	4.09	0.024*
Width (cm)	108	*(81)*	178	*(185)*	87	*(28)*	1.81	0.176
Depth (cm)	16	*(7)*	20	*(12)*	17	*(10)*	1.02	0.369

Table 2.6 Mean physicochemical characteristics of Macaronesian streams flowing through different land use types. Continuous variables only analysed. Standard deviation is shown in italics. *F* ratios (41 degrees of freedom) and *p* values for one-way ANOVA are given. Significance is indicated: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

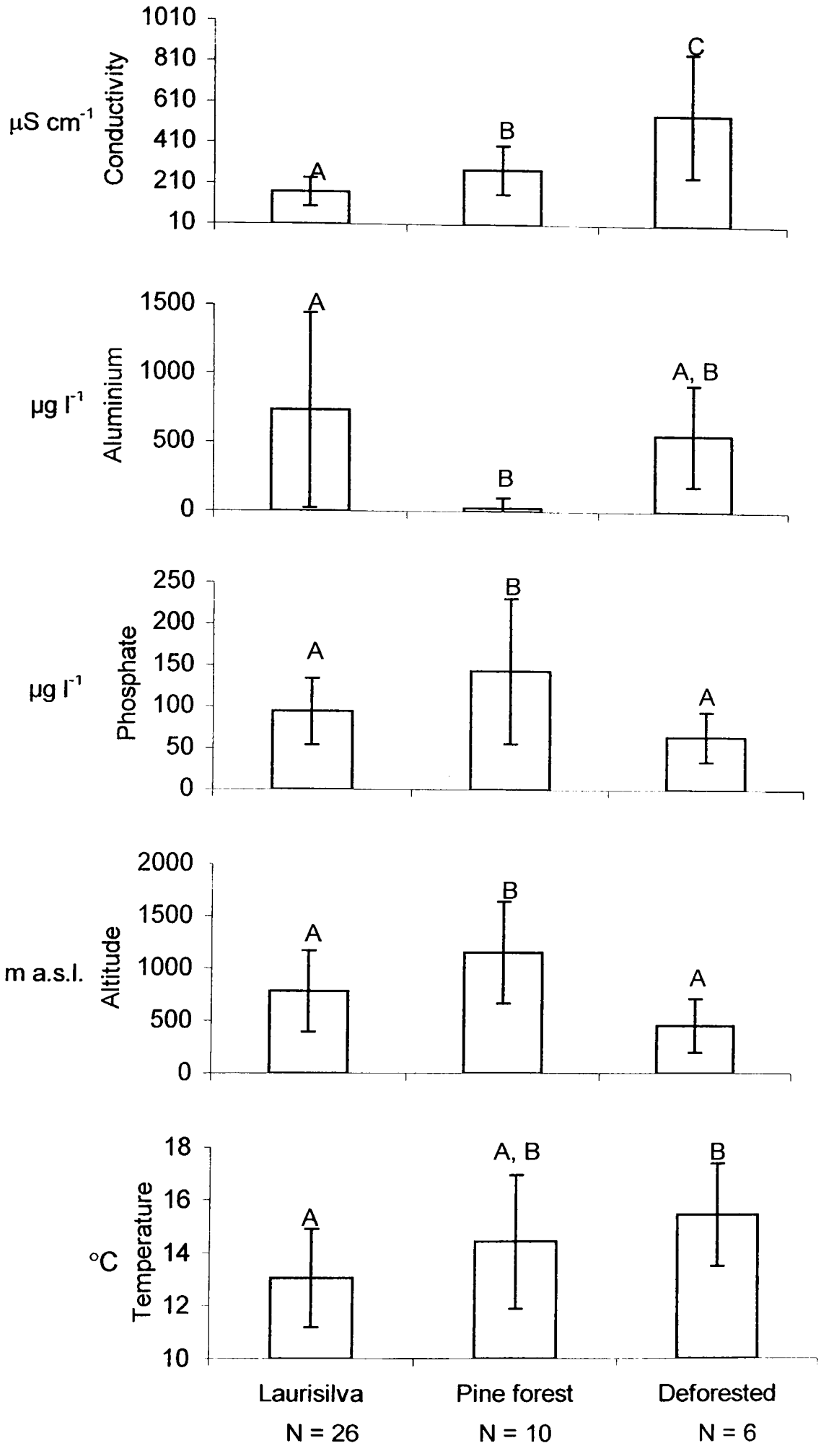


Figure 2.8 Physicochemical variations between Macaronesian streams flowing through three land use types. Lettering as in Figure 2.7.

2.4 Discussion

The aims of this chapter were to document the physicochemistry of the Macaronesian streams, and to investigate differences between islands and land use types in terms of stream physicochemistry. The PCA of water chemistry data showed that there were no over-riding chemical gradients across the streams, which were generally poor in ions other than aluminium (*i.e.* sites were not arranged along an axis dominated by any one particular variable). The first two axes of the water chemistry PCA were dominated by the variables phosphate, hardness, aluminium, copper and zinc, whilst the first two axes of the physical PCA were dominated by altitude, temperature, width, depth, distance from source and cover of boulders, cobbles and rocks.

The islands differed significantly in terms of their water chemistry (specifically, pH, conductivity and aluminium). These differences were predicted to be closely related to island age through the process of weathering (Giller and Malmqvist, 1998). However, this was not the case: there were no trends of increasing ion concentration with island age (even when the Tenerife data was recalculated separately for areas of the island with different geological ages). Both the youngest island (La Palma) and a much older island (Madeira) had low conductivity. Geological differences between the islands that determine catchment bedrock may account for the differences, particularly in aluminium, which occurs at much higher levels on La Gomera and Madeira than on La Palma and Tenerife, and pH, which is low in streams on La Palma.

On average, Madeiran streams were at lower altitudes than Canarian streams. This is likely to reflect a much greater pressure on water resources on the Canary Islands than on Madeira (Rodríguez Brito, 1995). The Canaries have lower rainfall, as they are further

south and experience hot dry Saharan winds for part of the year. Combined with this, the efficient diversion of water from springs and stream beds into enclosed pipes on the Canary Islands has reduced the number and diversity of streams (Malmqvist *et al.*, 1993); those at high altitudes and with low flows are most likely to be untouched. Stream temperature varied significantly with altitude but was lowest on the low island of La Gomera, the island where the streams were the most densely shaded (80% being in *laurisilva*).

Streams flowing through catchments with different land uses also differed physicochemically. The trends in stream physicochemistry with land use are to a degree confounded by the differing frequencies of land use types on the islands. The significant difference in altitude between pine forest and *laurisilva* streams is related to altitudinal zonation of the land uses (Báez, 1979; Gandullo, 1991). For example, on the Canary Islands, pine forest occurs at higher altitudes than *laurisilva*, and deforested streams are found in areas that are more accessible and at lower altitudes. The lower water temperature recorded for *laurisilva* streams perhaps indicates a direct effect of land use on the stream environment, in the form of shading (Townsend *et al.*, 1997a; Giller and Malmqvist, 1998). Whilst shading was recorded, a statistical analysis of the variation in degree of shading with land use could not be performed within this study. Note also that water temperature (and other variables) were recorded on only one occasion for each stream, hence conclusions drawn are tentative rather than absolute statements.

Conductivity also differed significantly between land use types, probably due to a combination of factors including altitude and disturbance, as land uses typically occur at different altitudes and in varying proximity to mankind's activities. The higher conductivity of deforested streams may be due to increased erosion, nutrification where

streams flow through agricultural land (Hughes, 1997), geological differences, and/or a non-significant trend of increasing conductivity with decreasing altitude. Aluminium was significantly lower in pine forest streams than in *laurisilva* streams. This may be a result of the low aluminium found overall on La Palma, where most of the pine forest streams were situated, as coniferous catchments are usually associated with high levels of aluminium (Ormerod *et al.*, 1993), in part due to the association of this vegetation with increased soil and water acidity (Rutt *et al.*, 1990). Pine forest streams also differed from others in their phosphate concentration: again, this could be an effect of the unbalanced replication of land use types across the islands, as all the pine forest streams occur on La Palma and Tenerife, islands with high mean stream phosphate. Alternatively, phosphate concentrations may reflect the ability of the catchment vegetation to absorb and retain the phosphates, or may reflect flow regime, concentrations becoming elevated in streams with low discharge (Mackay, 1995; Giller and Malmqvist, 1998).

The physicochemistry of streams on Tenerife has been surveyed previously (Malmqvist *et al.*, 1993); some temporal variation in pH and conductivity was found, likely to be caused by the rainfall regime (Hornung and Reynolds, 1995; Giller and Malmqvist, 1998). Indeed, stream physicochemistry may show diel as well as seasonal variation. The results of the current study are therefore qualified by the fact that, due to logistical constraints, point measurements only were taken; hence, only robust patterns in the data are discussed. Hughes (1995, 1997) found high levels of similarity in water chemistry among Madeiran streams, as did this study, with a transition in the data set from low to high conductivity, mineral content and temperature. The position of sites on this continuum related to potential enrichment from agriculture and habitations and to distance from their source.

Finally, the data were examined for extreme ion concentrations likely to exert large influences on the distribution of stream invertebrates. Comparison with data collected by Gower *et al.* (1994) on Cornish metal-polluted streams suggests that aluminium was the only metal present in potentially toxic concentrations (> 0.2mg l^{-1}, Giller and Malmqvist, 1998) in Macaronesian streams. For comparison, in Welsh upland streams, mean aluminium concentrations ranged between 0.04mg l^{-1} and 0.48mg l^{-1}, whilst acidity ranged between pH 6.9 and pH 4.6 (Rutt *et al.*, 1989; Rundle and Ormerod, 1991). Nearly one third of the Canarian streams had aluminium concentrations greater than 0.4mg l^{-1}, however all of the streams sampled were circum-neutral (pH > 6.3); experimental studies have suggested that aluminium is not a significant toxicant at the concentrations and pH occurring in these streams (Sutcliffe and Hildrew, 1989). Gower *et al.* (1994) found that copper had the greatest effect on community composition; in the present study, copper concentrations were low (< 0.006mg l^{-1}) except at P1, G1, G2 and M4 (0.006 – 0.025mg l^{-1}). Aquatic insects have a higher toxic threshold for zinc (≈ 0.1mg l^{-1}), and zinc concentrations in the Macaronesian streams were low (< 0.04mg l^{-1}). It was therefore predicted that no strong faunal gradients with metal ion concentrations would be found.

Chapter 3

A Hierarchical Analysis of Macaronesian Stream Invertebrate Community Composition

A Hierarchical Analysis of Macaronesian Stream Invertebrate Community Composition

SUMMARY

Species richness trends and faunal variation, on regional to local scales, were investigated in the macroinvertebrate fauna of Macaronesian island streams. Inter-island variation in freshwater invertebrate species richness and community composition is investigated for the first time, allowing the study of the effects of island properties, such as age, isolation and water chemistry, on the fauna. Species and family presence/absence and abundance data were obtained from a quantitative and qualitative sampling scheme encompassing 42 streams across four islands.

At the largest scale, species richness differed significantly between islands (Chi squared, $p < 0.001$), as did mean species richness per stream, both of the total macroinvertebrate fauna (ANOVA, $p < 0.001$) and of individual orders (ANOVA, all $p < 0.01$). Local (stream) species richness was significantly correlated with regional (island) richness ($p < 0.02$), being a constant 41-49% of the regional species pool. Island species richness tended to increase with island area, altitude and age, and to decrease with isolation. Community composition in terms of the transformed abundances of the taxa present, at both species and family level, also differed significantly between islands (ANOSIM, $\rho < 0.001$) - La Palma and Tenerife were the only island pair between which community composition did not differ significantly. Inter-site relationships, in terms of community composition at species and family levels, were significantly correlated (ANOSIM, $\rho < 0.001$).

At the catchment scale, the three land use types (*laurisilva*, pine forest and deforested land) differed in mean stream species richness (ANOVA, $p < 0.001$) but not in total richness. Deforested streams had significantly more species than streams in other land use types. The difference in community composition between pine forest and deforested streams was also significant (ANOSIM, $\rho < 0.001$).

At the local scale, stream species richness was significantly related to four physicochemical variables (calcium, magnesium, conductivity and pH) ($p < 0.01$). Community composition was related to stream physicochemical variables reflecting substratum composition, flow, shade, and water chemistry (co-efficient = 0.411). Different variables were important on different islands. The abundance of selected common species also varied significantly with a variety of physicochemical variables. Generally, these variables differed from those that varied significantly with island and land use type (Chapter 2). Thus, significant variation in community composition was found at all scales, from islands through catchment land use type to individual stream characteristics.

3.1 Introduction

A community can be defined as an assemblage of actually, or potentially, interacting species, or as a spatial, functional or taxonomic association of species (Schluter and Ricklefs, 1993). Communities have emergent (*e.g.* resilience to disturbance) as well as collective (*e.g.* species diversity) properties; however, they are usually regarded as a level of organisation rather than an entity (Begon *et al.*, 1996). Communities are not discrete, but their boundaries are often defined by habitat discontinuities, for example the species assemblage inhabiting running water at a locality (Minshall, 1988). The community structure is the result of the pattern of resource allocation among the species present and of

patterns of their spatial and temporal abundance (Cody and Diamond, 1975). Individual species respond to the environment in different ways, so characteristically different communities arise in different environments.

The notion that a single process, such as competition or predation, should be the fundamental determinant of community composition has proved to be too narrow (Hildrew *et al.*, 1984; Kohler, 1992; Ricklefs and Schluter, 1993b; Hugueny and Cornell, 2000). Community assembly is hierarchical, the product of both regional and local influences (Ricklefs and Schluter, 1993a; Poff, 1997; Rundle *et al.*, 2000), and it has been proposed that local communities are a subset of the regional species pool, determined by species passing through environmental and dispersal 'filters' (Belyea and Lancaster, 1999) (Section 1.1.2). Stream community assembly can also be investigated in terms of a spatial hierarchy: pools and riffles form reaches within streams, which are grouped into catchments, watersheds and regions.

The study of the influence of environmental variation, at a range of scales from the microhabitat to the catchment and beyond, has been developed into the habitat templet approach to understanding community assembly of freshwater invertebrates (Southwood, 1977; Frissell *et al.*, 1986; Townsend *et al.*, 1997b). This model involves predicting and testing associations of species traits with axes of environmental variation (*e.g.* temporal dispersal frequency with habitat disturbance frequency). Richards *et al.* (1997), using a regression-based approach, found that a number of both reach- and catchment-scale properties were highly predictive of species traits. Poff (1997) developed an alternative niche-based approach, describing species in terms of their functional relationships to habitat selective forces.

The Macaronesian islands offer an opportunity to investigate variation in community composition at several scales (Section 1.5.1), reflecting both environmental variation, which filters species through habitat availability and niche requirements, and nestedness of species pools, additionally determined by dispersal and historical biogeography (Figure 1.1; Chapter 4). The overall aim of this chapter was to investigate patterns in Macaronesian stream invertebrate communities at three spatial scales: island, catchment and stream reach. Differences in species richness and community composition between islands were tested and related to predictions from island biogeography (MacArthur and Wilson, 1967; Williamson, 1981; Gotelli and Graves, 1996).

Firstly, the four islands studied, La Palma, La Gomera, Tenerife and Madeira, differ in age, isolation, area and altitude, which are all predicted to determine species richness, with all but isolation showing positive correlations; such relationships have been described for terrestrial taxa on Macaronesian islands by Enghoff and Báez (1993) and Fernández-Palacios and Andersson (1993). Secondly, it was expected that local richness (mean species richness per stream) would be positively correlated with regional (island) richness, often the case in stream faunas, as for other biotas (Poff, 1997; Vinson and Hawkins, 1998; Griffiths, 1999; Malmqvist and Hoffsten, 2000). Thirdly, differences in species richness (due to the above patterns) were predicted to lead to significant differences in community composition between streams on different islands that would over-ride smaller scale influences from local physicochemical variation between streams *within* islands. The latter pattern would also be predicted to be influenced by the fact that the Macaronesian islands have a high level of endemism in their fauna, with many endemics restricted to only one island (*e.g.* Malmqvist *et al.*, 1995; Hughes *et al.*, 1998; Juan *et al.*, 2000). The fauna of Madeira is particularly differentiated from that of the Canaries, although the difference is reduced at higher taxonomic levels (*e.g.* family). The

faunal relationships between sites are expected to be similar, however, at species and family levels.

Although large-scale factors might be predicted to be of most importance in shaping stream communities, more local effects of land use, might be predicted to over-ride inter-island influences. For example, there is variation in allochthonous (*e.g.* detritus quality and timing of leaf fall) and autochthonous stream inputs (*e.g.* variation in algal production with shading) between land use types (*e.g.* Ormerod *et al.*, 1994; Abelho and Graça, 1996; Read and Barmuta, 1999). Hence, differences in species richness and community composition between land use types (*laurisilva*, *Pinus canariensis* forest and deforested catchments) were also investigated. It was hypothesised that *laurisilva* streams would be the most species-rich, due to the presence of *laurisilva*-specialist endemic species, including relictual palaeoendemics (*e.g.* Juan *et al.*, 2000) (Chapter 1), compared with pine forest and deforested streams.

Finally, local scale influences on species richness and community composition were inferred through examining correlations with environmental variables. Many studies have found that a wide range of physical and chemical stream properties are related to macroinvertebrate abundance and community composition. Significant variables may describe catchment topography and land use, stream size and permanence, substratum composition, marginal vegetation and water chemistry, particularly pH and aluminium (*e.g.* Vinson and Hawkins, 1998; Murphy and Giller, 2000; Malmqvist and Hoffsten, in press). The association between species distributions and stream characteristics is sometimes close enough that physicochemical data is a good predictor of the species to be found at a site (*e.g.* Minshall and Petersen, 1985, Minshall *et al.*, 1985) (Section 1.1.2). Within the Macaronesian islands and land use types, streams vary in characters such as

altitude, size, substratum composition and flow. Tests can be made for associations between the local stream environment and species richness (*e.g.* Zhang *et al.*, 1998: Malmqvist, 1999; Malmqvist and Hoffsten, 2000; Milner *et al.*, 2000) community composition (*e.g.* Hughes, 1995, 1997; Malmqvist *et al.*, 1997) and abundance of individual species (Willoughby and Mappin, 1988).

3.2 Methods

3.2.1 Faunal sampling and identification

The location and physical and chemical characteristics of the 42 study streams are described in Chapter 2. Faunal sampling was carried out in March-April 1998 (Canary Islands) and June 1998 (Madeira). Due to a number of practical constraints stream macroinvertebrate community sampling was performed during only one field season — the results present a 'snapshot' of the stream biota. However, the omission of the study of temporal variation in the stream communities allowed for a larger number of stream sites to be surveyed, and the invertebrates to be sorted and identified in detail.

The stream riffle fauna at each site was sampled quantitatively with a Surber sampler (area 0.125m^2, mesh size 1mm^2) (Surber, 1970). Five replicate samples, positioned randomly within the stream (without regard to faunal distribution but constrained by the small size of many streams), were collected from riffles, the dominant habitat, within one stream reach. A two-minute sample was also taken in a pool and a riffle at each site, using a hand net (23cm x 26cm frame, 0.5m-deep bag, mesh size 1mm^2) (Furse *et al.*, 1981); a hand search was also performed, in an attempt to ensure that all taxa were sampled. All samples were preserved individually in 100% ethanol on site. In the laboratory, they were stored at 4°C prior to being sorted through to remove all macroinvertebrates. These were

sorted initially to order and preserved in 70% ethanol. (Five replicate samples of meiofauna were also collected; however, work on meiofauna was outside the scope of the present study).

The following groups were identified to species where possible and enumerated: Amphipoda, Coleoptera, Ephemeroptera, Hemiptera, Mollusca, Odonata and Trichoptera. For certain taxa in these groups, identification to species level was not possible (Notes to Appendix 3.1). Diptera were identified to family level. Literature used for identification is listed in the additional bibliography. The abundance of each species was calculated for each site from the quantitative (*i.e.* Surber) samples by taking the arithmetic mean of the abundances in the replicate samples. Family abundance data was generated by summing the mean species abundance over all species in the family. Parsons and Norris (1996) suggest that community differences between sites can be adequately detected by sampling the riffle habitat alone, but qualitative (*i.e.* net and hand search) samples provided additional records for presence/absence matrices. It was assumed that the combination of sampling methods did not underestimate taxon richness (Clifford and Casey, 1992), whilst the short length of many of the streams ensured that the stream reaches sampled were representative of the whole stream (Minshall, 1988; Statzner and Borchardt, 1994; Clenaghan *et al.*, 1998).

3.2.2 Statistical analysis

A Chi squared test was used to test for variation in total species richness (calculated from presence/absence matrices generated from quantitative and qualitative samples) between islands and land use types. One-way analysis of variance (ANOVA) was used to test for significant differences in mean stream species richness (again derived from presence/absence matrices) between islands and land use types. A *post hoc* multiple range

test (least significant differences) was used to highlight significant differences between groups. The relationship between island (*i.e.* regional) and stream (*i.e.* local) richness was investigated using Pearson's product-moment correlation co-efficient. Correlations of species richness with island age, area, isolation (distance from nearest continent) and maximum altitude (a surrogate for habitat diversity) (data in Chapters 1 and 2), and correlations between stream species richness and physicochemistry, were similarly tested.

Multivariate analyses investigating differences in community composition between sites, and relationships between community composition and environmental variables, were performed using the PRIMER package of programmes (Plymouth Routines in Multivariate Ecological Research, Clarke and Warwick, 1994), analysing the quantitative samples. Before analysis, samples and species with zero abundance totals were removed from data matrices and the data fourth root transformed to down-weight the influence of dominant species (Clarke and Green, 1988; Clarke, 1993; Burton *et al.*, 2001).

To group streams according to their faunal similarities, CLUSTER (Clarke and Green, 1988) analysis was used to produce a dendrogram of inter-site distances. The method was of hierarchical agglomerative clustering of sites using group-average linking of a Bray-Curtis similarity matrix. No minimum number of sites per cluster was set, in order to avoid artificial groupings including distinct sites. MDS (Multi-Dimensional Scaling: Kenkel and Orlóci, 1986; Clarke, 1993; Clarke and Ainsworth, 1993) was used to corroborate the associations shown by cluster analysis. MDS comprised an ordination of the sites in a sample-space, according to relative values in the Bray-Curtis similarity matrix. The positioning of the points on the plot reflected the ranks of the pair-wise distances, rather than absolute distances. The stress of the plot was the distortion between the actual multidimensional similarity rankings and the corresponding distance rankings on

the plot. Ten restarts were used to ensure that global minimum stress levels had been reached in the ordination. SIMPER was used to identify which species accounted for the similarities of sites within clusters.

To test for a significant correlation between site similarity matrices based on species and family data, the RELATE routine was used. It tested the data against a null hypothesis of no correlation, using Spearman's rank correlation co-efficient in a randomisation test. That is, the co-efficient was recalculated 999 times with random reassignment of the site labels on one of the similarity matrices, and if the observed statistic exceeded that found in 95% of the simulations then the null hypothesis was rejected at the 5% level.

To test for differences in community composition between islands and land use types, one-way analyses of variance were performed with ANOSIM (Analysis of Similarities: Clarke and Green, 1988; Clarke, 1993). Analyses were performed on the matrix of Bray-Curtis similarities, with significance determined by a permutation test (999 permutations). SIMPER was used to identify which species accounted for the differences between islands and land use types.

The BIOENV programme (Warwick and Clarke, 1991; Clarke, 1993; Clarke and Ainsworth, 1993) was used to determine which combinations of the 22 physicochemical variables recorded for each stream best correlated with differences in community composition between streams. A similarity matrix of normalised Euclidean distances between sites generated from log-transformed standardised physiochemical data was related to the site similarity matrices generated from species data as above. The method compared the rank similarities of the biotic and abiotic data sets, calculating a Spearman's

rank correlation co-efficient. However, a significance value cannot be assigned to the co-efficient as the rank similarities are mutually dependent data points. A maximum of five explanatory abiotic variables was specified.

3.3 Results

3.3.1 Island-scale patterns

Species diversity of the Macaronesian streams (Appendix 3.1) differed between taxonomic groups and islands (Table 3.1). Coleoptera (34 species) and Trichoptera (19 species) were the most speciose groups and Tenerife (61 species) the most species-rich island, followed by La Gomera (38 species), La Palma (25 species) and Madeira (23 species). The four islands differed significantly in their total species richness (Chi squared test, $p < 0.001$), and in their richness of Coleoptera (Chi squared test, $p < 0.001$), compared to a null model of equal richness. Total richness is in each case that found in the present study; the sampling method did not allow for an estimate of the actual island faunal richness (that of Tenerife and Madeira is well known, Table 1.3).

Note that the number of permanent streams varies from island to island: the number of streams present may have a bearing on the total number of species recorded, for both statistical reasons (see below) and ecological reasons (*e.g.* increased quantity and diversity of habitat). In fact, the island with the fewest streams, Tenerife, is most species-rich. On Madeira, not all permanent streams were sampled but a number similar to the other islands were selected for comparison with the faunal richness of the Canary Islands. It is not necessary to standardise total species richness of the Canary Islands by stream number, as all suitable streams were surveyed (as opposed to a random sub-sample). One way in which the number of stream sampled has an effect on the number of species recorded is

Group	La Palma (N = 12)		La Gomera (N = 10)		Tenerife (N = 9)		Madeira (N = 11)		All	Mean richness		Island total
	Mean	Total	Mean	Total	Mean	Total	Mean	Total	Total	*F*	*p*	*p*
Coleo.	4.83	12	8.40	16	9.89	25	1.46	6	34	27.98	0.001	0.005
Amphi.	0	0	0.20	1	0	0	0	0	1	N/A	N/A	N/A
Ephem.	1.25	3	1.30	3	3.00	4	1.73	2	6	7.43	0.001	N/A
Hem.	0.83	3	2.20	4	2.33	6	0.55	1	8	16.73	0.001	N/A
Moll.	0.75	1	2.20	4	2.33	5	1.18	3	7	4.84	0.006	N/A
Odon.	0.58	1	0.20	2	3.00	9	0.18	2	9	9.85	0.001	N/A
Trich.	2.17	5	4.00	8	4.56	12	4.91	9	19	9.13	0.001	0.400
All groups	**10.41**	**25**	**18.5**	**38**	**25.11**	**61 1**	**10.01**	**23**	**84**	**18.13**	**0.001**	**0.001**
Unique species	N/A	1	N/A	4	N/A	23	N/A	18	46	N/A	N/A	0.001

Table 3.1 The species richness of macroinvertebrate groups in streams on four Macaronesian islands. N is number of streams. Sampling effort was constant across streams. 'Mean' is mean species richness per stream on each island, *i.e.* the mean of the richness recorded in each stream (*not* the total richness divided by the number of streams); 'Total' is total species richness on each island; 'Unique species' is the number of species found only on one island, in the present survey. The significance of variation in mean stream species richness (ANOVA, 41 degrees of freedom), and island total species richness (Chi squared test), is given. Chi squared test performed where expected values were greater than or equal to five. Stream species richness data: Appendix 3.2.

through increasing the number of individuals sampled per island. Figure 3.1, however, shows that the cumulative species richness levels off for each island (associated with the nestedness of the fauna, Chapter 4); more species would only be found by increasing sampling effort within streams. Further investigation of the data showed that streams with intermediate densities of individuals had the highest species richness (Figure 3.2); increased sampling effort in streams with relatively low and high macroinvertebrate densities is unlikely to yield new species. The sampling scheme is not appropriate for a rarefaction analysis of estimated increase in species number with increased sampling effort.

Madeira was particularly poor in Coleoptera, whilst Tenerife was richer in Ephemeroptera and Odonata than the other islands (Table 3.1). The islands also differed in the number of single-island species present on each (as recorded in the present survey) (Chi squared test, $p < 0.001$), compared to an equal number of single-island species; in particular, 18 of 23 species on Madeira were not found on the Canary Islands, many of these being Madeiran endemics. A high proportion of species (17%) were found at only one site, and the Tenerife stream fauna included the majority of single-site species, in addition to 23 species not found on other islands.

Differences between islands in the richness of individual streams were also significant, for both the total fauna (ANOVA, $p < 0.001$) and individual taxonomic groups (ANOVA, $p < 0.006$ in every case) (Table 3.1). A multiple range test showed that La Palma and Madeira did not differ in mean species richness but that La Gomera and Tenerife were distinct, with Tenerife containing all six streams with over 20 species (Appendix 3.1). Mean species richness per stream (local richness) was significantly correlated with island (regional) species richness ($R^2 = 96.58\%$, $p < 0.017$), and was a

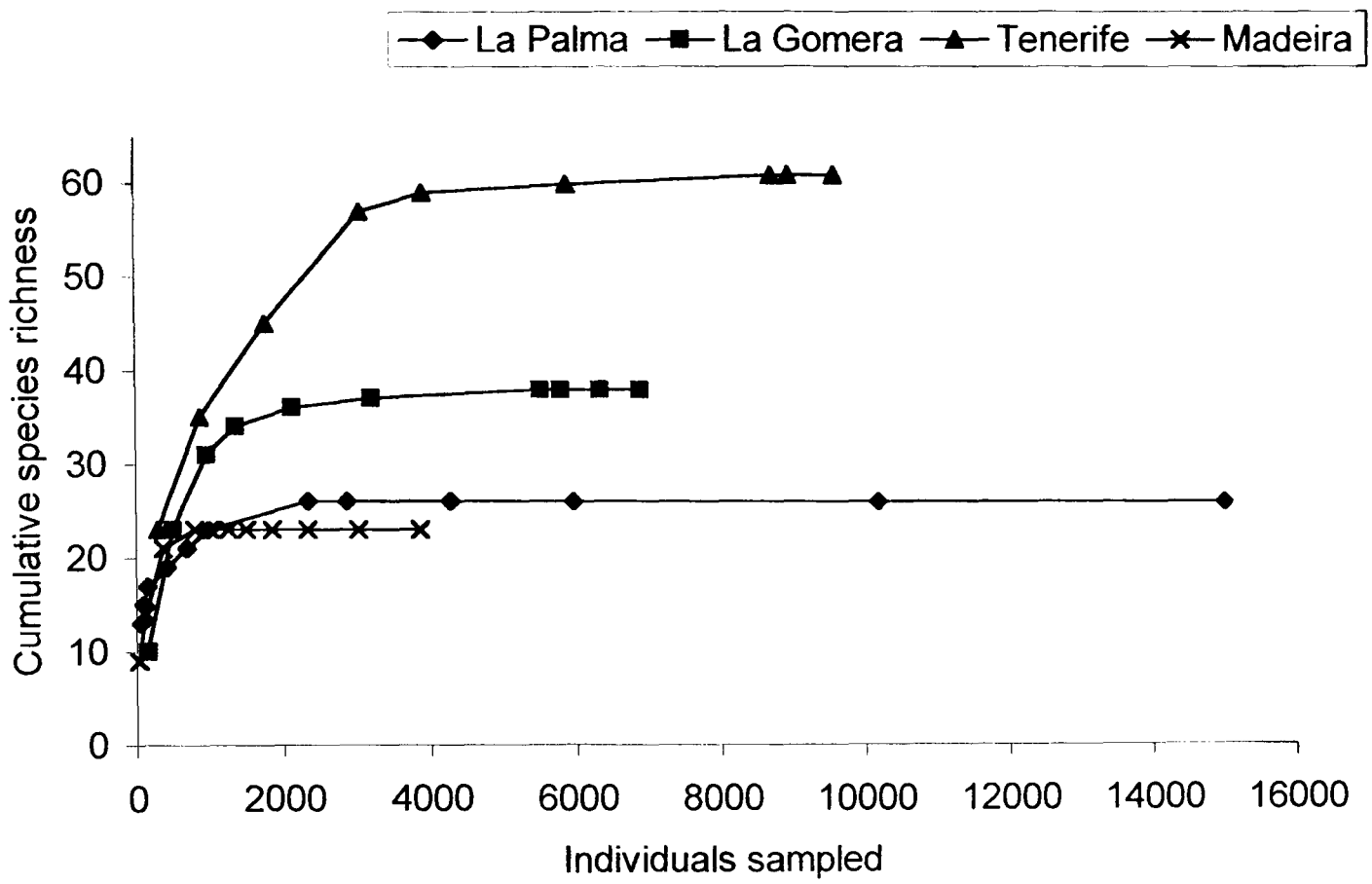


Figure 3.1 Cumulative species richness plotted against number of stream macroinvertebrates sampled for four Macaronesian islands. Between nine and twelve streams surveyed per island, including all the permanent streams on La Palma, La Gomera and Tenerife. Points added to plot in order of number of additional species per number of individuals captured by standard sampling method.

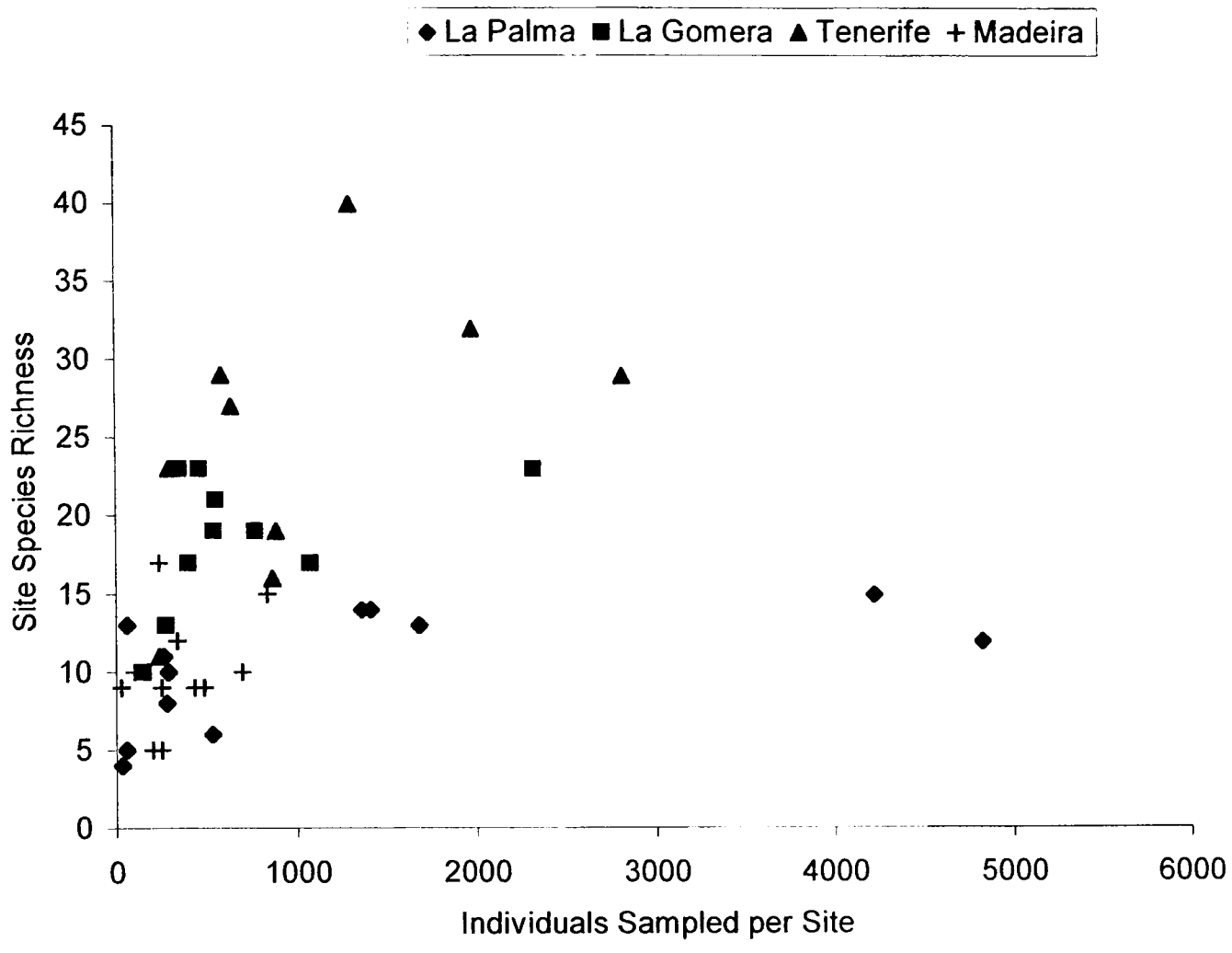


Figure 3.2 Number of macroinvertebrate species plotted against number of individuals found at Macaronesian stream sites. Sampling effort was standardised across streams.

relatively constant proportion (41-49%) of the island species pool (Figure 3.3). Within taxonomic groups, the mean richness per stream was a more variable proportion of the island species pool (Table 3.1). Mean species richness per stream on the islands was comparable with that for Gran Canaria (11 to 36 species) (Nilsson *et al.*, 1998).

The sizes of island species pools (and consequently mean species richness per stream) were not significantly correlated with island age, area, and distance from nearest continent or maximum altitude. Though high regression co-efficients were obtained, the relationships may not be linear (Table 3.2; Figure 3.4).

Finally, considering differences between stream communities on the four islands, faunal assemblages differed significantly at both species (ANOSIM, $\rho < 0.001$, global $R = 0.71$) (Table 3.3; Appendix 3.3) and family level (ANOSIM, $\rho < 0.001$, global $R = 0.455$) (Appendix 3.4). All pairs of islands were significantly different (ANOSIM, $\rho < 0.05$), except for La Palma-Tenerife, whether species or family data were used. The grouping of streams by island, with Madeiran streams being particularly distinct, is illustrated by the MDS plot and concordant CLUSTER diagram (Figures 3.5 and 3.6); five distinct groups of sites occurred at the 40% similarity level. The MDS plot is a good representation of the relative similarities between faunal assemblages, having low stress. Table 3.4 lists the indicator species identified by SIMPER for each of the five site clusters. The clusters were characterised by their average abundances of 19 different species. It was not possible to test the distribution of sites from different islands in the clusters against a null model of an equal number from each island in each cluster group. However, groups did appear to be related to islands: group 1 is exclusively Madeiran streams; group 2 is a particularly species-poor stream on La Palma; and groups 3-5 are Canarian streams dominated by three different species: *Dryops gracilis* (Coleoptera: Dryopidae), *Baetis canariensis*

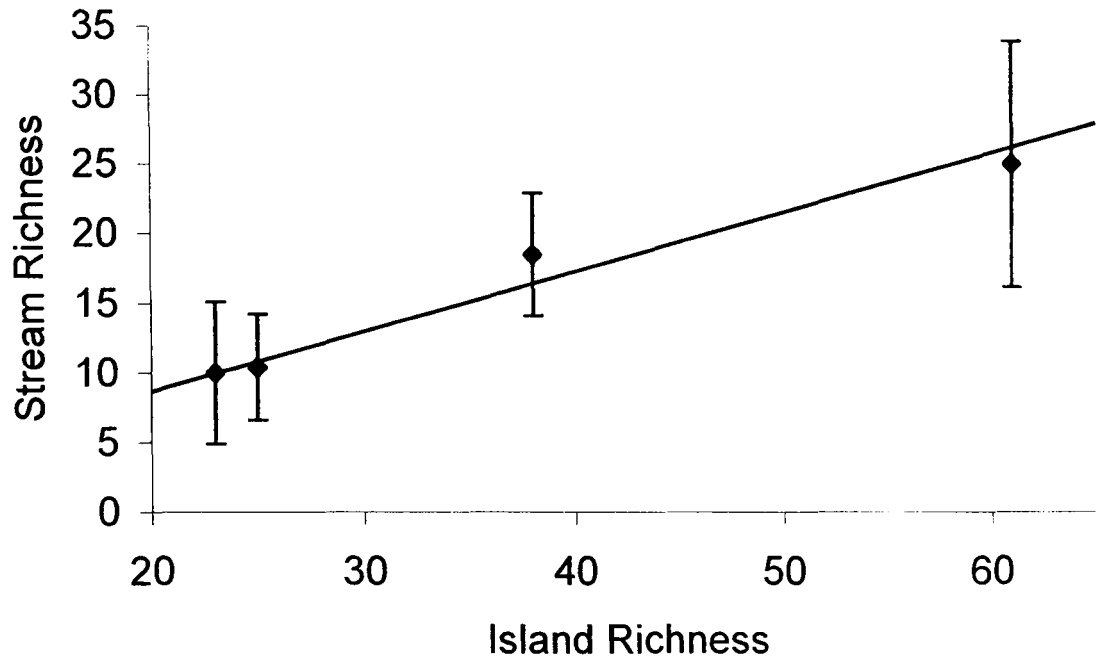
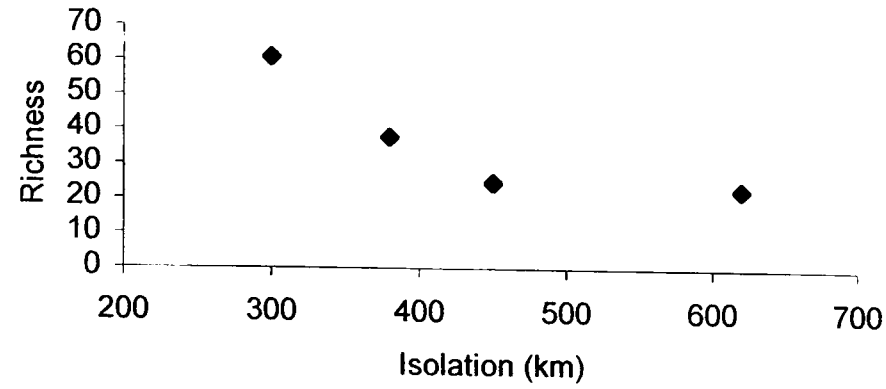
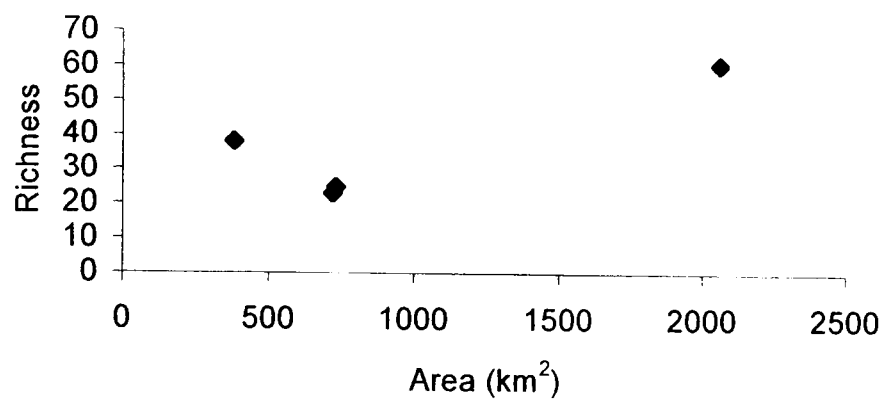


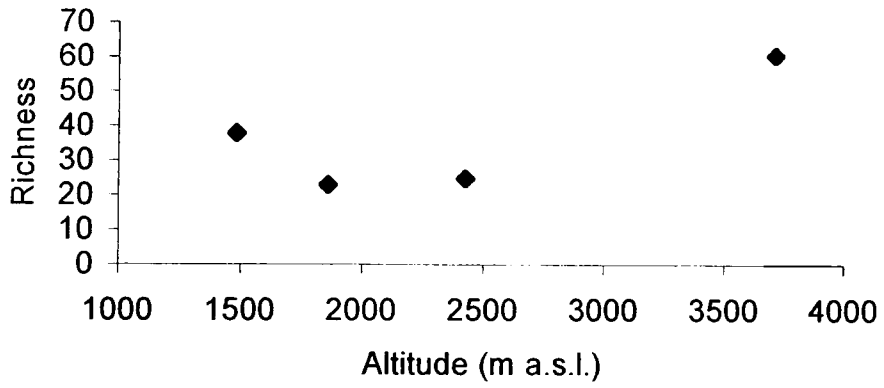
Figure 3.3 The relationship between mean stream and island species richness in the Macaronesian freshwater invertebrate fauna. (Pearson's product moment correlation, R^2 = 96.58%, $p < 0.017$). Standard deviation of mean stream richness is shown. Island and stream richness are that found in the present study.

Factor	*r*	*p*
Isolation	-0.849	0.151
Area	0.820	0.180
Altitude	0.740	0.261
Age	0.579	0.421

Table 3.2 The relationship between stream macroinvertebrate species richness and island characteristics of four Macaronesian islands. Island data: Chapter 1. Pearson's product-moment correlation co-efficient and *p* value of the linear regression model are given.







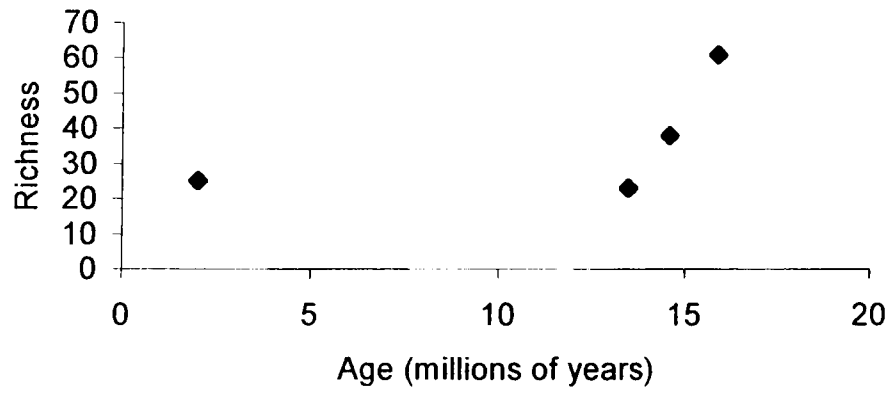


Figure 3.4 Stream macroinvertebrate species richness and island characteristics of four Macaronesian islands. No significant relationships were found.

Table 3.3 Taxa contributing to dissimilarity in macroinvertebrate communities between four Macaronesian islands. Taxa (see Notes to Appendix 3.1) are more abundant in the island heading the column than in the island heading the row. Taxa are listed within cells in order of their contribution to the dissimilarity (cumulative total 50%).

	La Palma	La Gomera	Tenerife	Madeira
La Palma	N/A	*Dryops gracilis* *Wormaldia taganananа* *Ancylus striatus* *Baetis pseudo./nigrescens* *Velia lindbergi* *Oecetis* sp.	*Dryops gracilis* *Ancylus striatus* *Mesophylax aspersus* *Hydropsyche* sp. *Baetis pseudo./nigrescens* *Laccobius canariensis*	*Baetis rhodani* *Tinodes* spp. *Hydropsyche maderensis* *Baetis pseudorhodani*
La Gomera	*Hydroptila* spp. *Baetis canariensis*	N/A	*Hydroptila* spp. *Baetis canariensis* *Hydropsyche* sp. *Mesophylax aspersus* *Ancylus striatus*	*Baetis rhodani* *Tinodes* spp. *Hydropsyche maderensis* *Hydroptila* spp. *Baetis pseudorhodani*
Tenerife	*Hydroptila* spp. *Baetis canariensis*	*Wormaldia taganananа* *Baetis pseudo./nigrescens* *Agabus biguttatus* *Oecetis* sp. *Hydraena serricollis* *Velia lindbergi*	N/A	*Baetis rhodani* *Tinodes* spp. *Hydropsyche maderensis* *Baetis pseudorhodani*
Madeira	*Hydroptila* spp. *Baetis canariensis*	*Dryops gracilis* *Ancylus striatus* *Wormaldia taganananа* *Mesophylax aspersus*	*Baetis canariensis* *Dryops gracilis* *Baetis pseudo./nigrescens* *Ancylus striatus* *Hydroptila* spp. *Hydropsyche* sp.	N/A

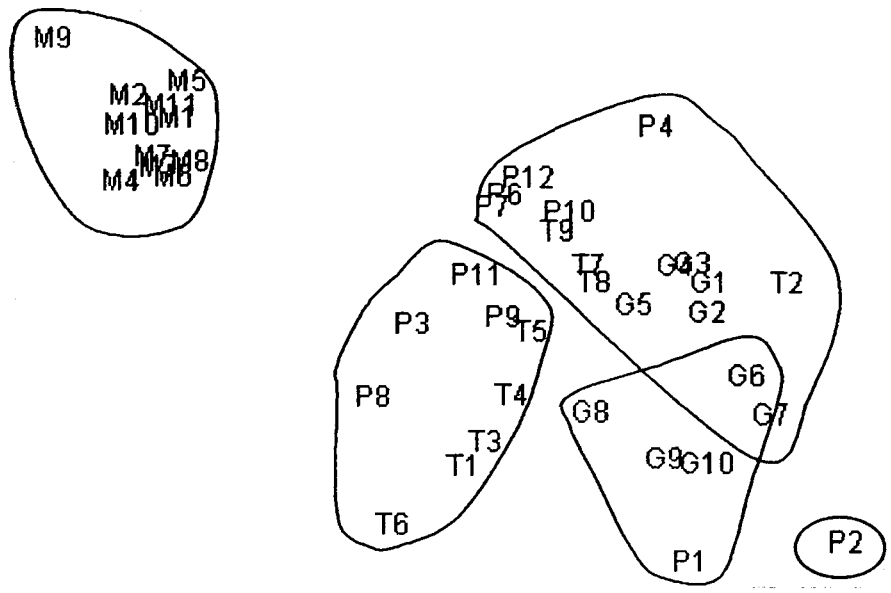


Figure 3.5 MDS plot of 42 Macaronesian streams ordinated by macroinvertebrate species abundance data. Stress = 0.11. Groups indicated correspond to those identified by cluster analysis (Figure 3.6). See Section 3.2.2 for explanation of method.

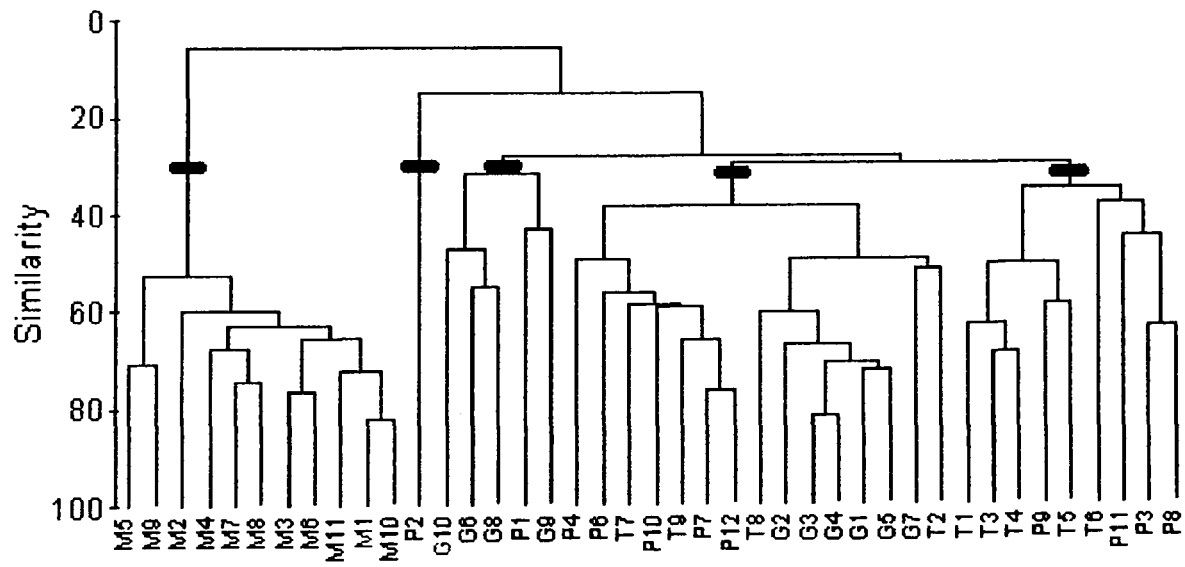


Figure 3.6 CLUSTER diagram of 42 Macaronesian streams, grouped by macroinvertebrate species abundance data. Groups numbered 1-5 from left to right; 30-40% similarity within groups; minimum group size not specified. See Section 3.2.2 for explanation of method.

Table 3.4 Taxa contributing to dissimilarity in macroinvertebrate communities between five groups of Macaronesian streams identified by cluster analysis (Figure 3.6). Taxa are arranged as in Table 3.3.

	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	N/A	*Ancylus striatus*	*Dryops gracilis* *Ancylus striatus*	*Baetis canariensis* *Baetis pseudo./ nigrescens* *Mesophylax aspersus* *Hydroptila* spp.	*Hydroptila* spp. *Dryops gracilis* *Ancylus striatus* *Laccobius canariensis*
Group 2	*Baetis rhodani* *Tinodes* spp. *Hydropsyche maderensis*	N/A	*Dryops gracilis* *Hydraena serricollis* *Pisidium casertanum*	*Baetis canariensis* *Baetis pseudo./ nigrescens* *Mesophylax aspersus* *Hydroptila* spp.	*Hydroptila* spp. *Dryops gracilis* *Ancylus striatus* *Laccobius canariensis*
Group 3	*Baetis rhodani* *Tinodes* spp. *Hydropsyche maderensis* *Hydroptila* spp. *Baetis pseudorhodani*	*Ancylus striatus* *Velia lindbergi*	N/A	*Baetis canariensis* *Baetis pseudo./ nigrescens* *Hydroptila* spp. *Mesophylax aspersus* *Wormaldia tagananana*	*Hydroptila* spp. *Ancylus striatus* *Laccobius canariensis* *Baetis canariensis* *Hydropsyche* spp. *Nebrioporus canariensis*
Group 4	*Baetis rhodani* *Tinodes spp.* *Hydropsyche maderensis* *Hydroptila* spp.	*Ancylus striatus*	*Dryops gracilis* *Ancylus striatus* *Pisidium casertanum*	N/A	*Hydroptila* spp. *Laccobius canariensis* *Ancylus striatus* *Dryops gracilis*
Group 5	*Baetis rhodani* *Tinodes* spp. *Hydropsyche maderensis* *Baetis pseudorhodani*	*Velia lindbergi*	*Dryops gracilis* *Pisidium casertanum* *Mesophylax aspersus*	*Baetis canariensis* *Baetis pseudo./ nigrescens* *Mesophylax aspersus* *Agabus biguttatus*	N/A

(Ephemeroptera: Baetidae) and *Hydroptila* species (Trichoptera: Hydroptilidae). At higher similarity levels, within groups 3-5, the clustering of streams by island breaks down.

The analysis was repeated for family data (Figures 3.7 and 3.8). Again, Madeiran streams grouped together, though the Madeiran group was not as distinct from Canarian streams as it was at species level. Similarity of streams within islands was significant (see above), and is apparent from Figure 3.6. As a result, RELATE revealed a significant correlation ($\rho < 0.001$, global $R = 0.532$) between the site similarity matrices produced by species and family data.

3.3.2 Mesoscale patterns

As with islands, different catchment land use types had different species richness and community composition (Appendix 3.2; Table 3.5). Total species richness did not differ significantly between land use types, though mean stream richness did (ANOVA, $p < 0.001$). Deforested streams were particularly rich in Odonata (9 species), and *laurisilva* streams in Trichoptera (16 species). A multiple range test showed that individual deforested streams had, on average, significantly more species than pine and *laurisilva* streams ($p < 0.05$), which were not distinct. Differences in total richness between land use types were significant for all taxonomic groups examined, with the exception of Trichoptera (ANOVA, $p < 0.02$). Differences between land use types in terms of the richness of endemic and non-endemic species are investigated in Chapter 4.

Differences in community composition between *laurisilva*, pine forest and deforested streams were not globally significant (ANOSIM, $\rho > 0.05$), but those between pine and deforested streams were (ANOSIM, $\rho < 0.001$). Pine forest streams supported higher mean abundance of *Baetis canariensis* and *Mesophylax aspersus* (Trichoptera:

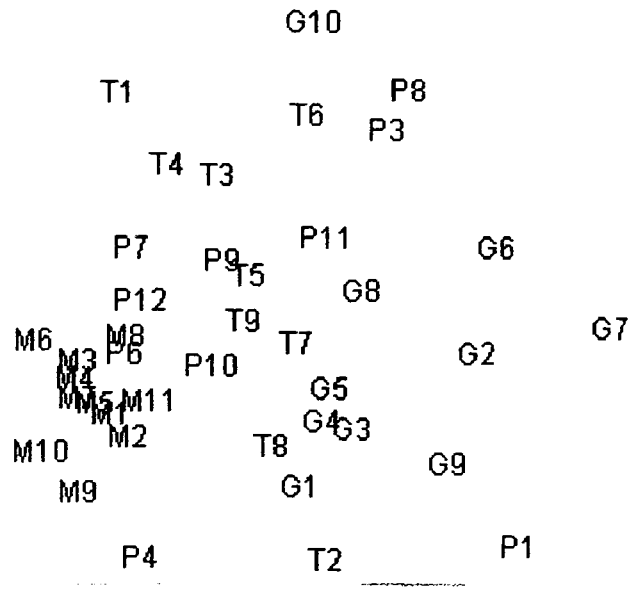


Figure 3.7 MDS plot of Macaronesian streams ordinated by macroinvertebrate family abundance data. Stress = 0.22. In this case, clear clusters of sites were not identified (Figure 3.8). See Section 3.2.2 for explanation of method.

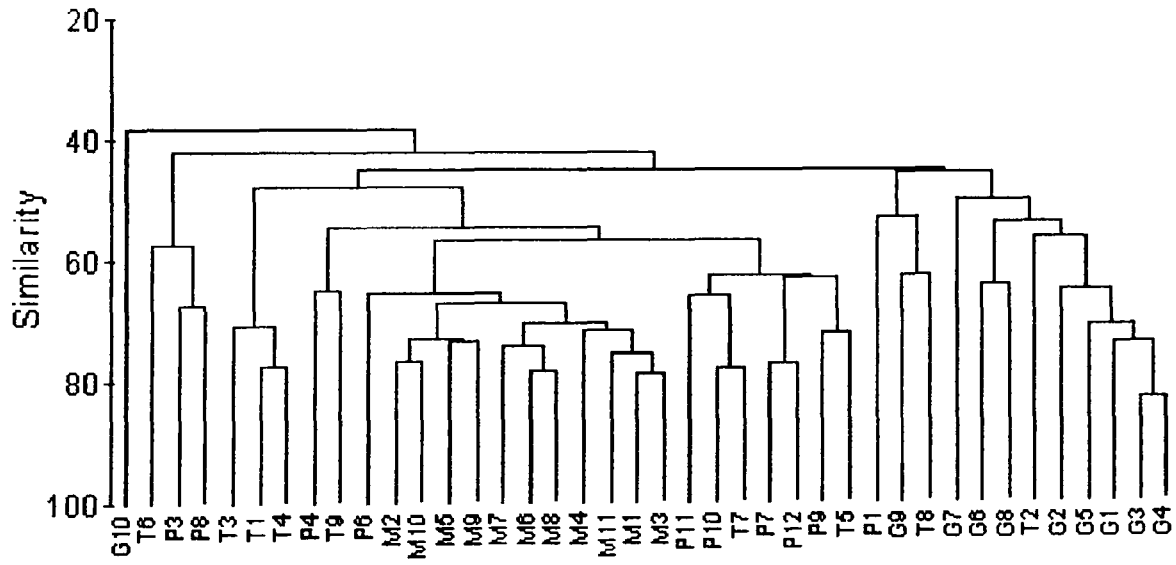


Figure 3.8 CLUSTER diagram of Macaronesian streams, grouped by macroinvertebrate family abundance data. See Section 3.2.2 for explanation of method.

	Laurisilva (N = 26)		Pine Forest (N = 10)		Deforested (N = 6)		Stream richness		Land use total
	Mean	**Total**	**Mean**	**Total**	**Mean**	**Total**	***F***	***p***	***p***
Coleoptera	4.54	22	7.33	21	8.33	22	6.01	0.002	0.985
Amphipoda	0.08	1	0	0	0	0	N/A	N/A	N/A
Ephemeroptera	1.42	5	2.08	4	2.5	6	4.93	0.005	0.819
Hemiptera	1.25	4	1.67	4	2.5	7	6.02	0.002	0.549
Mollusca	1.38	6	0.92	4	3.5	6	3.70	0.020	0.779
Odonata	0.21	2	1	5	3.5	9	6.75	0.001	0.100
Trichoptera	3.96	16	3.17	9	4.67	12	2.63	0.064	0.368
All groups	**12.38**	**56**	**15.67**	**47**	**25.0**	**62**	**7.35**	**0.001**	**0.355**
Unique species	N/A	14	N/A	4	N/A	11	N/A	N/A	0.067

Table 3.5 The species richness of macroinvertebrate groups in streams flowing through three land use types on the Macaronesian islands. 'Mean' is mean species richness per stream in each land use type; 'Total' is total species richness for each land use type. The significance of variation in mean stream species richness (ANOVA, 41 degrees of freedom), and island total species richness (Chi squared test), is given.

Limnephilidae), whilst deforested streams supported higher mean abundance of *Ancylus striatus* (Mollusca: Ancylidae), *Dryops gracilis* and *Hydropsyche* sp. (Trichoptera: Hydropsychidae) (Table 3.6). The CLUSTER analysis (Figure 3.6) also tended to group streams by catchment land use; however, it was not possible to test this statistically.

3.3.3 Local-scale patterns

Calcium, magnesium, conductivity and pH were significantly correlated with species richness across islands (Pearson's correlation co-efficient, $p < 0.01$) (Table 3.7; Figures 3.9 and 3.10). Richness increased with increasing calcium and magnesium ion concentrations and conductivity and decreased with increasing pH. Note that pH was not recorded for Madeiran streams and thus the relationship applies to Canarian streams only.

Stream physicochemistry also influenced community composition (in terms of transformed mean abundance of species in replicate Surber samples). Across all four islands, community composition was best explained by substratum type — a combination of boulder, rock and cobble cover gave a correlation co-efficient of 0.394 (Table 3.8). The co-efficients were higher when islands were considered individually. The factors that best explained faunal inter-site similarities differed from island to island: a variety of factors describing substratum composition, water chemistry, shading, flow and distance from source were important (Table 3.8).

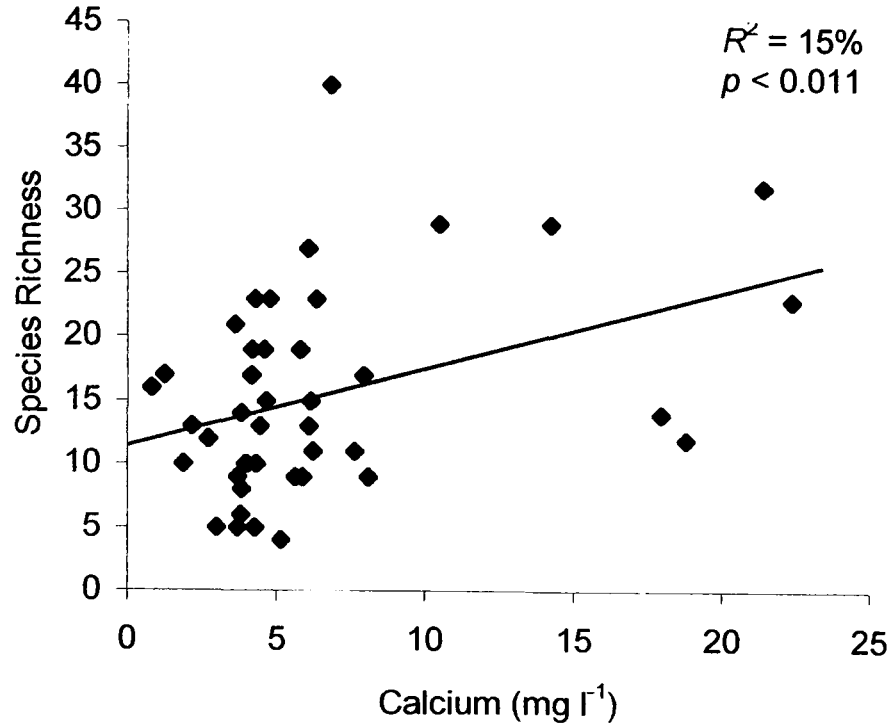
The abundance of eight of the ten most common species on the Canary Islands was correlated with at least one physicochemical variable (Table 3.9). Approximately eight significant correlations would be expected by chance (due to the size of the matrix): 19 were found, indicating that species' abundances relate to at least some environmental variables. A mixture of positive and negative trends in abundance with increasing ion

	Laurisilva	Pine	Deforested
Laurisilva	N/A	*Hydroptila* spp. *Baetis canariensis* *Baetis pseudo./nigrescens* *Mesophylax aspersus* *Dryops gracilis* *Agabus biguttatus*	*Hydroptila* spp. *Ancylus striatus* *Dryops gracilis* *Hydropsyche maderensis* *Nebrioporus canariensis*
Pine	*Baetis rhodani* *Ancylus striatus*	N/A	*Ancylus striatus* *Hydroptila* spp. *Dryops gracilis* *Hydropsyche maderensis* *Lymnaea truncatula* *Physa acuta* *Nebrioporus canariensis*
Deforested	*Baetis rhodani* *Baetis canariensis* *Baetis pseudo./nigrescens* *Tinodes* spp. *Mesophylax aspersus*	*Baetis canariensis* *Mesophylax aspersus* *Baetis pseudo./nigrescens*	N/A

Table 3.6 Taxa contributing to dissimilarity in macroinvertebrate communities in Macaronesian streams flowing through three land use types. Taxa (see Notes to Appendix 3.1) are more abundant in columns relative to rows. Taxa are listed within cells in order of their contribution to the dissimilarity (cumulative total 50%).

Variable	*r*	*p* value
Altitude	-0.076	0.633
Source	0.211	0.181
Temperature	0.105	0.509
Width	-0.040	0.802
Depth	0.233	0.138
Copper	0.046	0.177
Zinc	0.189	0.230
Magnesium	**0.498**	**0.001**
Calcium	**0.389**	**0.011**
Aluminium	0.015	0.923
Phosphate	0.039	0.806
Iron	-0.063	0.691
Hardness	-0.059	0.710
Conductivity	**0.671**	**0.001**
pH	**-0.511**	**0.003**

Table 3.7 The relationship between Macaronesian stream macroinvertebrate species richness and physicochemistry. Pearson's product moment correlation co-efficient and *p* value of correlation are given. Effect of island statistically eliminated. Physicochemical data: Appendices 2.1 and 2.2; richness data: Appendix 3.1.



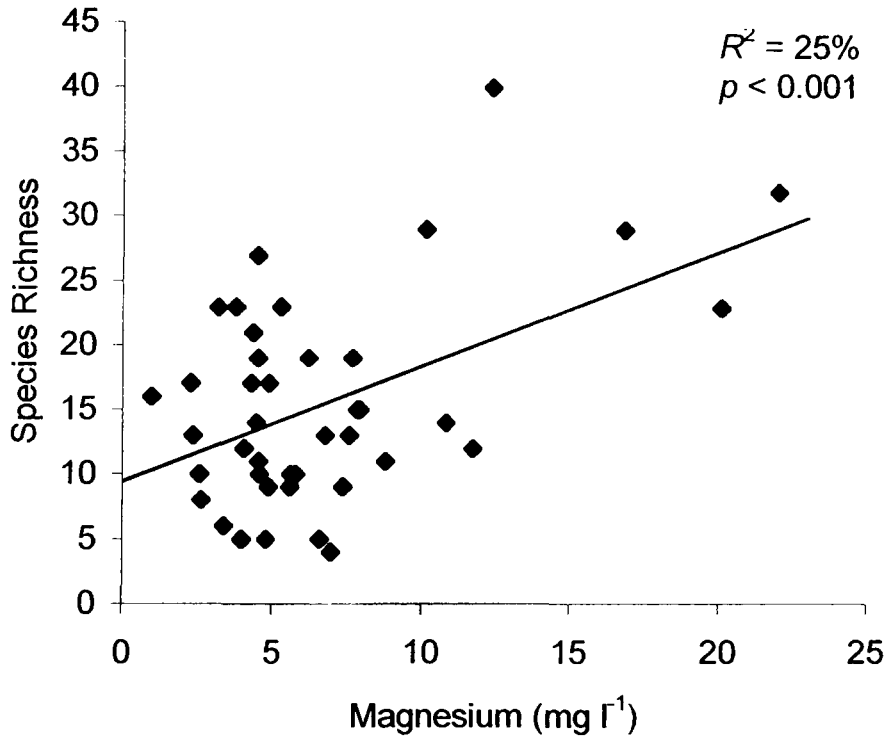
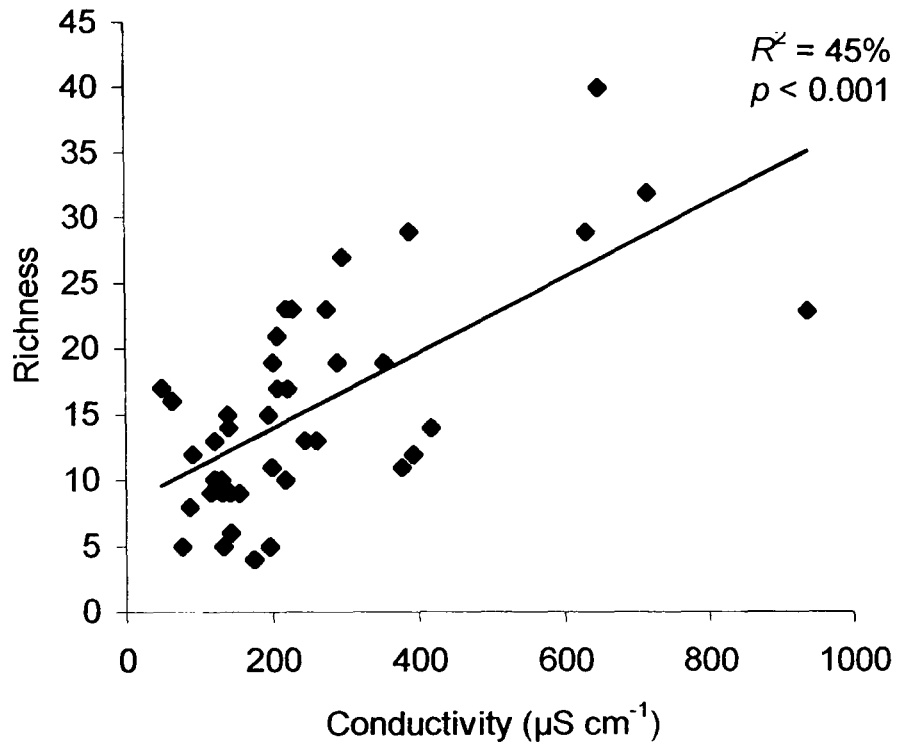


Figure 3.9 The relationship between calcium and magnesium ion concentrations and macroinvertebrate species richness in Macaronesian streams. Simple linear regression performed.



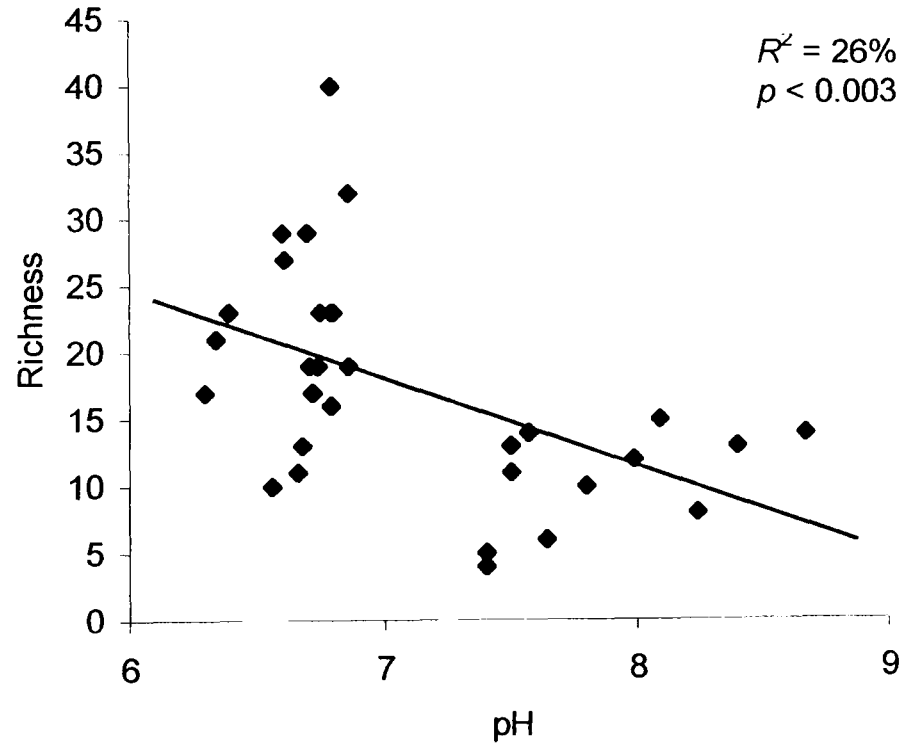


Figure 3.10 The relationship between conductivity and pH and macroinvertebrate species richness in Macaronesian streams. Simple linear regression performed.

Overall	
Variables	**Co-eff**
Boulders	0.353
Boulders + cobbles	0.384
Boulders + cobbles + rocks	**0.394**

La Palma	
Variables	**Co-eff**
Hardness	0.681
Gravel + hardness	**0.796**
Gravel + FPOM + hardness	0.794

La Gomera	
Variables	**Co-eff**
Iron	0.450
Source + phosphate	0.543
Source + shade + phosphate	**0.600**

Tenerife	
Variables	**Co-eff**
Flow	0.230
Flow + rocks	**0.344**
Flow + rocks + zinc	0.318

Madeira	
Variables	**Co-eff**
Hardness	0.238
Width + hardness	0.332
Flow + bedrock + hardness	**0.349**

Table 3.8 The relationship between Macaronesian stream macroinvertebrate community composition and physicochemistry. The correlation co-efficient is that between the species abundance-based site similarity matrix and the site similarity matrix based on the physiochemical variables listed. The highest correlations are shown in bold. Physicochemical data: Appendices 2.1 and 2.2; community data: Appendix 3.3.

Species	Significant Variables				
Agabus biguttatus (Dytiscidae)	Source* -				
Dryops gracilis (Dryopidae)	Width** -	pH* -			
Laccobius canariensis (Hydrophilidae)	Temperature** +	Conductivity* +	Magnesium* +		
Nebrioporus canariensis (Dytiscidae)	Copper* -				
Baetis canariensis (Baetidae)	Iron* -				
Velia lindbergi (Veliidae)	Temperature** -	Hardness* -	Aluminium* +	pH* -	
Ancylus striatus (Ancylidae)	Altitude** -	Depth** +	Source* +	Hardness* -	Magnesium* +
Mesophylax aspersus (Limnephilidae)	Zinc** -	Altitude** +			

Table 3.9 Relationships between the abundance of common macroinvertebrate species and physicochemistry of Canary Island streams. Pearson's product moment correlation co-efficient (above) and p value of correlation (below) calculated for ten species that are common on all three islands (La Palma, La Gomera and Tenerife). * $p < 0.05$, ** $p < 0.01$. Variables are listed in descending order of importance, and negative or positive correlations indicated. No variables were significant for *Limnebius gracilipes* or *Baetis pseudorhodani/nigrescens*. No species showed a significant correlation between abundance and calcium or phosphate (omitted from table).

concentration were found, for example, abundance of *Laccobius canariensis* (Coleoptera: Hydrophilidae) increased with increasing water temperature, whilst that of *Velia lindbergi* (Hemiptera: Veliidae) decreased with increasing temperature. In addition to temperature, distance from source, pH, conductivity, magnesium, hardness and altitude were important factors determining the abundance of more than one species.

3.4 Discussion

3.4.1 Island-scale patterns: biogeography and species pool characteristics

Tenerife was the most species-rich island, followed by La Gomera, La Palma and Madeira. Tenerife was predicted to have the most species under island biogeography models (McArthur and Wilson, 1967; Williamson, 1981; Gotelli and Graves, 1996; Losos, 1998; Ricklefs and Lovette, 1999), as it is the largest, oldest, least isolated and highest of the four islands studied. Whilst no significant correlation of species richness with these factors was found, due to the small number of data points, comparable data from additional North Atlantic islands are not available. Richness may tend to increase with increasing island area, altitude, age and proximity to the African continent, consistent with observations upon terrestrial taxa on the Macaronesian islands (Enghoff and Báez, 1993; Fernández-Palacios and Andersson, 1993).

Turning to the second hypothesis, local species richness, expressed as mean richness per stream, increased with regional species richness, as predicted. Again, this is qualified by the small number of data points (islands with permanently flowing streams) available, and the richness values apply only under the standardised sampling method used in this study and do not necessarily represent the total fauna. Island richness for the Canarian streams is the total from a sampling of *all* the permanent streams, whilst that for

Madeira is derived from sampling a similar number of streams (approximately one quarter of the total), allowing a fair comparison. The regional-local richness correlation has been suggested to be the norm for stream insects, as for other groups inhabiting island-like environments (Begon *et al.*, 1996; Poff, 1997; Vinson and Hawkins, 1998; Maurer, 1999). As stream richness appears to be dependent on regional (island) richness, the communities are likely to be unsaturated, because they are able to 'sample' a constant proportion of the island species pool, rather than dominated by inter-specific competition (assuming disturbance levels are low) (Caswell and Cohen, 1993; Hugueny and Cornell, 2000). The linear relationship between local and regional richness has been attributed to large-scale variation in climatic conditions and water chemistry (*i.e.* physicochemical variation on the regional scale): the regional species pool is reduced by passing through a strong environmental filter, and is then passively sampled by local species pools. In the present case, the isolation of the islands may also have prevented communities becoming saturated with species, because of the barriers to dispersal on partially arid, oceanic islands.

A strong influence of island and archipelago upon the faunal assemblages was found, in both univariate (richness) and multivariate (community) analyses. The fauna of Madeiran streams was distinct from that of Canarian streams due differences in the species pool: both the presence of Madeiran endemic species (*e.g. Hydropsyche maderensis*) and of more widespread species that were absent on the Canaries (*e.g. Baetis rhodani*). Within the Canary Islands, a clustering analysis of communities grouped species-rich and species-poor streams separately, and grouped separately streams dominated by *Dryops gracilis*, *Baetis canariensis* and *Hydroptila* species. Inter-island dissimilarity tended to arise from differences in the species pool in comparisons including Madeira, that is, the patterns of presence/absence rather than abundance of species. Comparative data are unavailable as this is the first such study of stream faunas on oceanic islands.

At family level, the similarity between streams is influenced less by the distributional range of taxa and consequently reflects ecological similarities between streams to a greater degree. However, at this level all island pairs, other than La Palma-Tenerife, still had significantly different stream communities. This suggests that biogeographic patterns in community composition reflect processes occurring at this taxonomic level: for example, dispersal ability is likely to be autocorrelated within families.

3.4.2 Mesoscale patterns: variation with land use

In contrast to predictions, deforested streams had significantly more species than *laurisilva* streams. Significant differences in community composition occurred between pine and deforested streams, due to differences in both abundance and presence/absence, but not between other land use combinations. Microhabitat diversity and palatability of organic matter may be important factors determining the pattern of species richness and community composition with land use (Pringle *et al.*, 1988; Malmqvist and Eriksson, 1995), and different functional feeding guilds may dominate streams in different catchment land use types (Yule, 1996). Finally, stream macroinvertebrate communities in the three land use types may differ in their invasibility. In deforested streams, higher levels of disturbance may enable more species to become established (as expressed by the intermediate disturbance hypothesis), whereas the more unchanging environment of the *laurisilva* and pine forest streams makes the stream communities more resistant to invasion by new species (Begon *et al.*, 1996). The types of disturbance likely to occur in the Macaronesian streams include disturbance by human activity (recreation, agriculture and road construction) in the deforested streams, and more severe variation in flow and temperature, on both diel and seasonal scales, in the deforested and pine forest streams

where catchment vegetation is sparser than in *laurisilva*. Invasibility generally decreases as a community develops, enabled by a predictable environment (Law, 1999).

Stronger effects of land use on species richness and community composition might have been found if land use types were classified in more detail (Richards *et al.*, 1997), and the effects were not confounded by local physicochemistry and the uneven distribution of land use types between the islands (Weatherley *et al.*, 1993). Malmqvist *et al.* (1993) suggest that, because of the lower richness and taxonomic diversity of Canarian compared to continental streams, communities are unsaturated and competition is relaxed, resulting in fewer habitat specialists. Conversely, the observed patterns of species richness and community composition with land use may be due to the absence of non-endemic generalists (*e.g.* Odonata) from *laurisilva* and pine forest streams, their being more abundant in deforested streams.

3.4.3 Local-scale patterns: variation with physicochemistry

As predicted, at the local scale stream species richness and community composition were related to the physicochemical nature of the streams. Stream species richness was correlated with four water chemistry variables (calcium, magnesium, conductivity and pH), but not with any physical variables, although species richness has been correlated with physical habitat diversity in some cases (Malmqvist and Eriksson, 1995). Species richness decreased with increasing pH in the Macaronesian streams, in contrast to previous studies of stream communities (Townsend *et al.*, 1983; Sutcliffe and Hildrew, 1989; Giller and Malmqvist, 1998).

Within islands, the correlations between community composition and physicochemistry were high: the species assemblage within a stream is not a random

subset of the island species pool. Different physicochemical variables correlated with the species similarity matrix on different islands. This may in part be due to differences in the species pool of the islands, as taxa are likely to respond to environmental conditions in different ways. In addition, streams varied in physicochemical gradients between islands. The most important correlates with community composition (in terms of the abundances of species present), both across and within islands, related to substratum composition, organic matter, shade, flow and water hardness. Flow and substratum type, together, may exert an influence on the community structure through the availability of flow refugia, potentially independently of stream channel size or morphology (Lancaster and Hildrew, 1993; Statzner and Borchardt, 1994). Substratum composition is also linked to habitat complexity (Hildrew and Giller, 1994) and flow to oxygen availability. Water chemistry was also important in some cases; species may respond to water chemistry directly (through tolerances in ion exchange mechanisms) or indirectly (through its effect on primary production, predators and prey), therefore the response would be expected to differ between the distinct communities on different islands (Sutcliffe and Hildrew, 1989; Mason, 1996; Vinson and Hawkins, 1998).

The number of correlations observed between abundance of particular common species and physicochemical variables is evidence that individual species show trends in abundance with stream physicochemistry, leading to the *overall* variation in species richness and community composition with physicochemistry. A wider range of variables than expected gave significant correlations, with those commonly being reported in the literature as being associated with species distributions, for example pH (Willoughby and Mappin, 1988) and aluminium (Mason, 1996), not appearing more frequently in correlations than others. The data also give clues to the ecological requirements of individual species; for example, *Velia lindbergi* may be a cold-water specialist, as may

Mesophylax aspersus, a species whose abundance increased with altitude. In contrast, *Ancylus striatus* is more abundant at stream sites that are larger (deeper), at lower altitude and further from the stream's source.

Two additional factors have been found to introduce spatial and temporal variation in previous studies of stream community composition. The first is longitudinal variation, summarised as the river continuum concept (Vannote *et al.*, 1980; Minshall and Petersen, 1985; Giller and Malmqvist, 1998) (Section 1.1.2). Spatial patterns in macroinvertebrate community composition are sometimes explained well by longitudinal variation in physical factors (Statzner and Borchardt, 1994; Clenaghan *et al.*, 1998); however, in this study all streams were first or second order so environmental and faunistic changes along the stream continuum should not confound the results. Secondly, seasonal variation in macroinvertebrate community composition may be pronounced (Minshall *et al.*, 1985; Malmqvist and Eriksson, 1995; Clenaghan *et al.*, 1998), determined by duration of life history stages, behaviour, climate, flow regime and patch disturbance frequency (Hildrew and Giller, 1994; Grimm, 1994). However, non-seasonal life cycles are more common in tropical climates than in the temperate zone (Wallace and Anderson, 1984) and, as the Macaronesian islands do not experience a strongly seasonal climate, the stream fauna has been shown to be relatively constant (Stauder, 1991; Malmqvist *et al.*, 1993), with some exceptions (Hughes, 1997). In the present study all sites were sampled in the same season, therefore temporal variation is not invoked.

3.4.4 Importance of processes at different scales

This study is the first investigation of freshwater faunal composition on the islands over the range of scales from regional (island and archipelago) to local (individual streams). Any combination of processes at the three hierarchical levels (island, land use

and stream environment) may play a part in determining stream community composition (*e.g.* Ormerod *et al.*, 1994). In determining the relative importance of factors acting at the different scales, where no significant relationship is found between environmental variables and community composition, a community may be randomly assembled or determined by larger scale factors. Conversely, if local scale factors are of the most importance, stream communities are expected to group together according to their physicochemical characteristics irrespective of island or land use. For example, in Welsh upland streams a number of taxa were influenced by forest management in the surrounding catchment despite an over-riding influence of stream acidity (Weatherley *et al.*, 1993). Note that the above is not exclusive of grouping by island or by land use, as both may affect local physicochemistry.

In this case, stream species richness, community composition and abundance of individual species varied with physicochemistry, and physicochemistry differed significantly between islands, but not land use types (Chapter 2). The effects of physicochemistry on the fauna can be distinguished from the effects of island biogeography in the case where those variables that vary with island are not those with which the community composition correlates. Indeed, the environmental factors that differed between islands (conductivity, aluminium, altitude, temperature, width and depth) appeared to be different to those that correlated with community composition or species richness within islands (water chemistry and substratum composition). The exception is conductivity: across all four islands, species richness increased with increasing conductivity.

Differentiation between stream communities from different land use types may be a response of the fauna to the varying conditions and energy inputs of streams in different

land use types, or perhaps an artefact of the significant differentiation between islands and the uneven representation of land use types on the islands. As the land use types did not differ significantly in terms of physicochemistry, if the organisms were responding to land use type independently of stream physicochemistry or island, then this would be a response to a factor not quantified in this study. For example, streams flowing through different land use types may differ in terms of nitrate levels, disturbance and flow regimes and daily temperature fluctuations.

The species pool on each island is a result of historical (dispersal) and evolutionary events as well as ecological filters (Enghoff and Báez, 1993; Malmqvist *et al.*, 1997). That the fauna does not respond strongly to those factors (width/depth and altitude/temperature) that strongly differentiate islands suggests that ecological filters are relatively unimportant in determining the island species pool. Corroborative evidence comes from other studies, in continental situations, where macroinvertebrate assemblage composition *was* strongly correlated with stream size (width/depth) and/or altitude/temperature (*e.g.* Delucchi, 1988; Corkum, 1989; Ormerod *et al.*, 1994).

It is therefore concluded that regional- (island) and local- (stream) scale processes combine to determine Macaronesian macroinvertebrate communities. In addition to characteristics of the stream itself, catchment land use may also exert an influence on the stream fauna. Considering stream communities within Tenerife, Malmqvist *et al.* (1993) suggested that small differences in the abiotic descriptors of different streams underlay the species distribution patterns they observed, in combination with dispersal-related factors (for example, stream isolation). Whilst the importance of stream physicochemistry in determining species richness and community composition has demonstrated, larger scale

factors, such as the regional species pool, determined by dispersal and evolution, are also important.

Chapter 4

Macroecological Patterns in the Macaronesian Stream Fauna

Macroecological Patterns in the Macaronesian Stream Fauna

SUMMARY

Macroecological patterns in the freshwater macroinvertebrate fauna of the Canary Islands and Madeira were investigated, in order to test hypotheses about evolutionary and ecological influences on community composition.

At the largest scale, biogeographic patterns in the fauna were investigated with the cladistic approach parsimony analysis of endemicity (PAE). It was demonstrated (Section 3.3.1) that the faunas of the four islands studied differed significantly, in both species richness and community composition. PAE elucidated the faunal relationships between the islands, showing close faunal similarity between La Gomera and Tenerife within the Canary Islands, and Madeira to be quite distinct.

Heterogeneity was found in the response of individual species to environmental variation (Section 3.3.3); further evidence for non-random distribution of species was provided by the detection of significant nestedness ($T = 20.37°C$, $p < 0.001$), taxa present at species-poor sites being subsets of the taxa at more species-rich sites. This is likely to be due to species differing in factors, such as degree of habitat specialism or dispersal ability, that affect their local colonisation and extinction probabilities.

Following the observation of significant variation in species richness between islands and land use types (Chapter 3) variation in richness of endemic and non-endemic species was similarly investigated. The number of endemic species differed significantly between islands (Chi squared test, $p < 0.005$); Tenerife had the greatest number of

endemics (22 species); however, the most isolated island, Madeira, had the highest proportion (73% of fauna was endemic). Richness of endemics also differed significantly between land use types (Chi squared test, $p < 0.002$); *laurisilva* streams contained more endemics (35 species) than pine forest or deforested streams (18 and 23 species, respectively).

For all taxa, and endemic taxa, there was no correlation between stream occupancy and abundance, but a significant positive relationship was found for the non-endemics. Endemic species had significantly higher occupancy than non-endemics (Wilcoxon test, $p < 0.024$), suggesting a greater habitat availability (number of streams suitable for colonisation) for the former. However, there was no significant difference in the abundance of endemics and non-endemics, suggesting broad similarity in niche widths.

These analyses demonstrated that, in addition to the island biogeographical variables, catchment land use and stream physicochemistry investigated in Chapter 3, there are two other important influences on the faunal communities. Firstly, there is an historical biogeographical effect due to the nature of the island study system, and secondly, there is an effect of inter-specific heterogeneity in factors, such as dispersal ability, niche width and habitat availability. These factors determine species' colonisation, local abundance and extinction at a site; there is a degree of systematic variation in the above between endemic and non-endemic species.

4.1 Introduction

4.1.1 The macroecological approach

The macroecological approach developed from recognition that regional patterns and processes can be important in determining the structure and dynamics of local assemblages (Ricklefs, 1987; Ricklefs and Schluter, 1993a, b; Gaston and Blackburn, 2000). This approach integrates ecological data at the population, community and ecosystem levels with evolutionary biology and biogeography to make statistical investigations of the distribution and abundance of organisms (Brown, 1995; Maurer, 1999). It also recognises that there may be patterns in, or constraints upon, species distributions at scales larger than the local one of traditional community ecology. These approaches treat species as anonymous, interchangeable units (Lawton, 1999), in contrast to the preceding chapter. Emergent properties of assemblages, such as richness trends, the linking of abundance with occupancy, nestedness and biogeographical patterns give a top-down approach to ecology, developing understanding of how individual communities are assembled (Brown and Lomolino, 2000a). Testable hypotheses can be constructed to draw conclusions about processes, such as dispersal and colonisation, that occur over scales too large to be studied directly (Gotelli and Graves, 1996).

Several studies have documented macroecological patterns among the biota of the Macaronesian islands (Section 1.3). Species richness and island area, age, isolation or habitat diversity have been found to be significantly correlated in some groups (*e.g.* Báez, 1992; Borges, 1992; Enghoff and Báez, 1993) and similar trends were found in the Macaronesian stream fauna (Chapter 3). Fernández-Palacios and Andersson (1993) concluded that faunal assemblages were the product of deterministic factors, dispersal ability and habitat availability. Malmqvist *et al.* (1997) investigated biogeographic patterns

and nestedness in Canarian Ostracoda, inferring dispersal ability and probability of successful colonisation from species' distributions. Faunal evolution and phylogenetic relationships have also been extensively studied in the Canarian herpetofauna for example (*e.g.* Brown and Thorpe, 1991; Thorpe, 1991; Thorpe *et al.*, 1996) and ecological (resource use), biogeographic and phylogenetic data were combined in a study on millipede species swarms (Enghoff and Báez, 1993).

Considering the Macaronesian stream fauna, within islands community structure correlated with stream physicochemical conditions, and the composition of the species pool varied significantly from island to island, the latter probably reflecting large-scale evolutionary and biogeographic processes (Chapter 3). In the following sections, four areas of investigation for stream invertebrates are introduced: overall relationships between island species pools, determined with parsimony analysis of endemicity (PAE); nestedness of stream faunas; variation in endemic species richness with island and land use type; and finally, the relationship between occupancy and abundance in endemic and non-endemic species.

4.1.2 Parsimony analysis of endemicity

Area cladograms produced by parsimony analysis of presence-absence matrices can be informative about biogeographical relationships between areas of endemism (*i.e.* areas of non-random distributional congruence among different taxa) (Morrone, 1994; Ron, 2000; Rundle *et al.*, 2000). The method has advantages over cladistic/vicariance biogeographical analyses in that it does not require prior knowledge about phylogenetic relationships of taxa within the fauna. In PAE, a cladistic parsimony analysis is performed with the sampling localities (streams or islands) as 'taxa' and species presences and absences as 'character states' (Rosen, 1988; Ron, 2000). Shared taxa are analogous to

synapomorphic character states in traditional cladistic analysis (Cracraft, 1991; Harvey and Pagel, 1991). PAE is most effective when distribution patterns are generated by vicariance, rather than sympatric speciation, by long-distance dispersal or by random local extinction events (Cracraft, 1994).

In the present context, the technique provides a method to generate specific hypotheses about the relationships between the freshwater faunas of the Macaronesian islands, for example, hypotheses about the direction of colonisation (Ron, 2000; Rundle *et al.*, 2000). Localities that appear most similar share a more recent history of faunistic exchange (Rosen, 1988) or indicate failure of allopatric speciation (Cracraft, 1991). PAE was performed on species presence/absence data to determine the overall faunal relationships between islands and to highlight which species distributions were responsible for those relationships. It was hypothesised that the cladogram would reflect the relative age, isolation or habitat composition (proportion of *laurisilva* streams) of the four islands (Figure 4.1), and that endemic species would be disproportionately represented amongst those species (shared taxa) discriminating nodes on the cladogram.

4.1.3 Nestedness

Several studies of faunas in insular habitats have revealed a pattern of 'nested subset' structure where more species-poor biotas contain a non-random subset of the species in richer biotas (Patterson and Atmar, 1986; Patterson, 1990; Whittaker, 1998). Nestedness is expected to be most pronounced in communities which are largely determined by the process of local extinction, for example in the case of biotic relaxation after habitat fragmentation, especially amongst groups which are poor dispersers (Patterson and Atmar, 1986; Patterson, 1987). However, it has also been demonstrated in

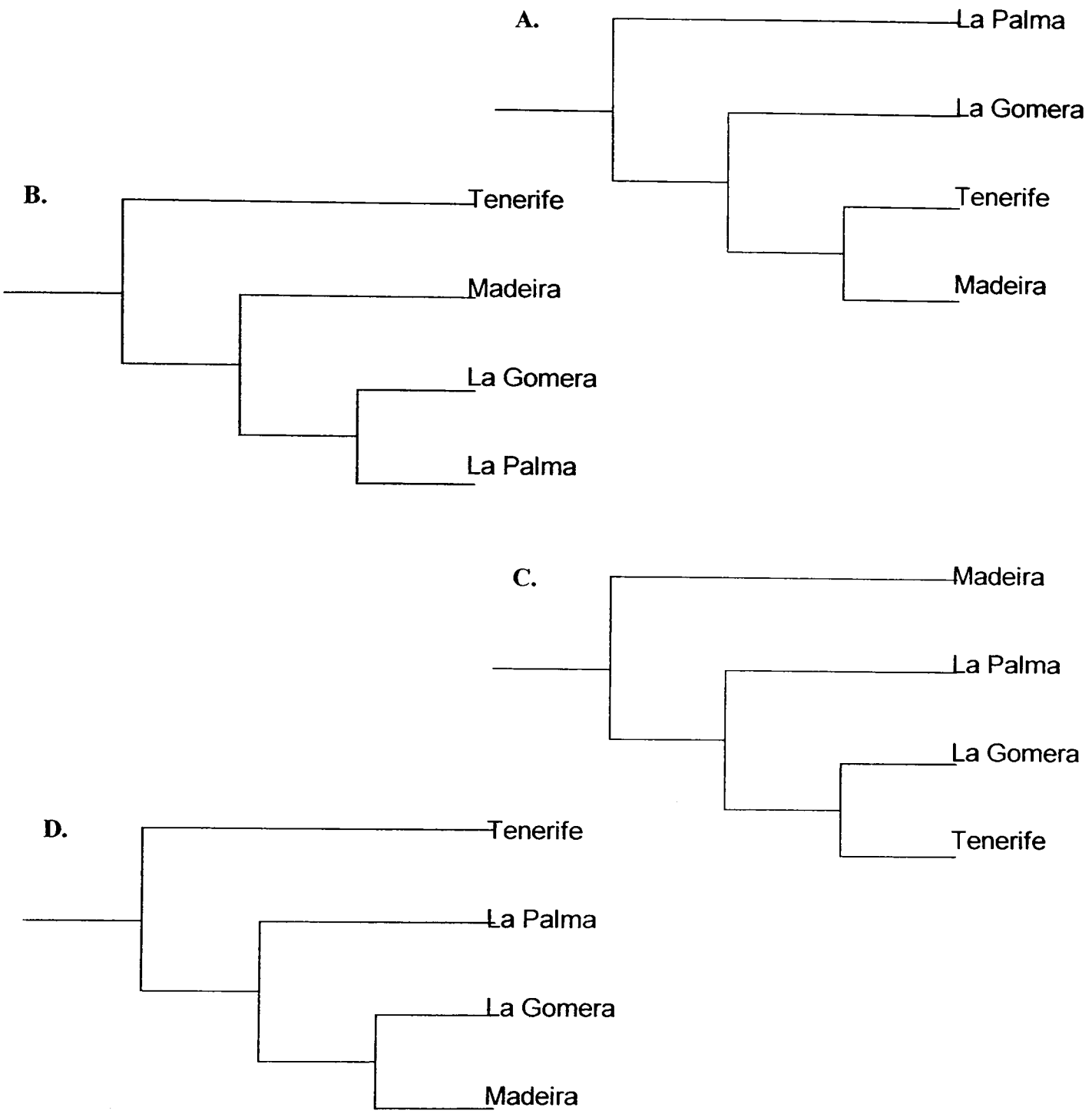


Figure 4.1 Hypothesised relationships between the macroinvertebrate stream faunas of four Macaronesian islands. A: relationships reflect island geological age, with the oldest islands most similar, and younger, more depauperate islands distinct, having experienced less colonisation. B: relationships reflect island geological age, with the youngest islands most similar, and older islands distinct due to increased allopatric speciation. C: relationships reflect island isolation, islands in closer proximity being more similar, due to inter-island dispersal. D: relationships reflect habitat availability, with islands having most *laurisilva* streams being most similar.

colonisation/dispersal-dominated communities (Patterson, 1990; Lomolino, 1996). In data sets structured by processes operating over historical time-scales, extinction will tend to be the process producing nestedness, whilst in a data sets structured over longer time-scales, colonisation will be the relevant process (Whittaker, 1998).

Nestedness is an emergent property of a suite of species, but results from the non-random distributions of individual species. Nestedness in a regional fauna has conservation implications, as all species can be protected by the most species-rich localities in the case of perfect nestedness (Patterson and Atmar, 1986; Patterson, 1987; Atmar and Patterson, 1993). However if only small fragments of habitat are preserved, the species within them after relaxation are likely to be the most abundant, generalist species - those in least need of protection (Patterson, 1987).

Whilst Boecklen (1997) generalised that aquatic invertebrates are an exception to this pattern, there have been few studies made to test this assertion; those of Nilsson and Svensson (1995) and Malmqvist and Hoffsten (2000) did find nestedness in freshwater faunas. However a greater number of studies have failed to find nestedness (*e.g.* Nilsson *et al.*, 1994; Malmqvist and Eriksson, 1995; Malmqvist *et al.*, 1997), or found variable responses among taxa (Malmqvist, 1999). The data are tested against the conventional null hypothesis of no nestedness.

4.1.4 Trends in species richness with endemicity

Patterns and causes of species diversity are a fundamental area of ecological investigation (Maurer, 1999) that can be studied at different spatial and temporal scales (Brown, 1995), and are a major component of macroecological research (Gaston and Blackburn, 2000). Species richness in Macaronesian streams is affected by both

environmental and biogeographic constraints. Stream invertebrate species richness has been related to physicochemistry (Malmqvist and Eriksson, 1995; Malmqvist and Hoffsten, 2000), and, in this case, island and catchment land use type (Chapter 3). At larger scales, biogeography plays an important part in determining how many species are present at each site (Vinson and Hawkins, 1998), particularly because of the presence of archipelago-specific endemics, and, more rarely, single-island endemics (Malmqvist *et al.*, 1995; Hughes *et al.*, 1998; Juan *et al.*, 2000). For example, species richness of endemics is expected to increase more steeply with island area than does richness of non-endemics, because of the greater opportunities offered by larger islands for adaptive radiation and speciation (Whittaker, 1998).

In this study, it was hypothesised that richness of endemic and non-endemic species would vary with island, with a greater proportion of the fauna being endemic on islands that were either older, larger or more isolated, due to the greater opportunities these conditions afford for endemism to evolve (Cox and Moore, 1993; Whittaker, 1998). However, the islands have different combinations of age, size and isolation (Chapter 1), so the null hypothesis tested was simply that the ratio of endemics to non-endemics is constant across the four islands of La Palma, La Gomera, Tenerife and Madeira.

Additionally, it was predicted that *laurisilva* streams would contain more endemics than streams in other land use types, and that more non-endemics would occur in deforested streams than in streams flowing through *laurisilva* or pine forest. This prediction was based on the assumption that endemic species are well adapted to *laurisilva* streams and less well adapted than non-endemic species to disturbed, deforested streams, and *vice versa*. Therefore, the second null hypothesis tested was that richness of endemics and non-endemics would be constant across land use types.

4.1.5 Occupancy, abundance and endemism

In the second area of investigation, species presence/absence records were analysed in relation to abundance data. The positive correlation between occupancy and abundance has been observed for several sets of 'ecologically similar' species, such as prairie grasses, bumblebees and United Kingdom farmland birds (*e.g.* Hanski, 1982a; Gotelli and Simberloff, 1987; Gaston and Lawton, 1989; Gaston, 1999; Maurer, 1999). In fact, the concept of correlated suites of traits, including abundance, habitat occupancy and geographic distribution, in sets of related species, was highlighted by Darwin (1859) (Brown, 1995). Several models have been put forward to try and explain this occupancy-abundance relationship (Table 4.1); these can be classified as static or dynamic, depending upon whether species' distributions and abundance are assumed to vary through time (Gotelli and Simberloff, 1987). Distinguishing between the various models with empirical data is problematic, however (Warren and Gaston, 1997; Hartley, 1998), due to the number of unrealistic assumptions required and the scale-dependency of the relationship (Collins and Glenn, 1997; Maurer, 1999; He and Gaston, 2000). Several of the models may contribute to an observed pattern: the existence of a number of mutually reinforcing, yet not necessarily independent, mechanisms behind the occupancy-abundance relationship may be typical of such macroecological generalisations (Gaston, 1996a, b; Gaston *et al.*, 1997b; Warren and Gaston, 1997).

Some previous investigation of the occupancy-abundance relationship has been made for freshwater communities, and positive correlations between occupancy and abundance were found (Malmqvist *et al.*, 1992, 1997, 1999; Hanski *et al.*, 1993; Nilsson *et al.*, 1994). The same relationship might be predicted in the Macaronesian stream fauna, but the high level of endemism (*circa* 50%) and the possible differential behaviour of endemic and non-endemic species sets under the above models (Table 4.1) may complicate

Model	Mechanism	Key References
Metapopulation dynamics	Inter-patch dispersal	Hanski, 1982a, b, c Hanski *et al.*, 1995
Dispersal ability	Varying dispersal ability	Hanski *et al.*, 1993
Niche breadth	Varying generalist-specialist strategies	Brown, 1984
Habitat availability	Varying habitat requirements	Venier and Fahrig, 1996
Density dependent habitat selection	Intra-specific competition	O'Connor, 1987
Geographical range structure	Limiting environmental gradients	Maurer, 1999
Density-independent responses	Varying population growth rates	Holt *et al.*, 1997
Taxon cycle	Speciation	Ricklefs and Cox, 1972
Phylogenetic non-independence	Spurious correlation due to sampling groups of related species having correlated traits	Gaston *et al.*, 1997b
Sampling effects	Spurious correlation due to difficulty of sampling rare species	Hanski *et al.*, 1993

Table 4.1 Theoretical models predicting a positive occupancy-abundance correlation.

predicted patterns. Endemism and rarity are closely allied at large scales but at local scales they are unconnected (Gaston, 1994) and so endemic species would not necessarily be expected to be rarer in terms of abundance or occupancy than non-endemics. However, endemics and non-endemics may differ with respect to their dispersal ability, habitat availability and niche breadth — three parameters relevant to several of the above models (Enghoff and Báez, 1993; Malmqvist *et al.*, 1997). These differences affect the predicted relative occupancy and abundance of the two sets of species.

Firstly, island endemics may tend to evolve traits that reduce dispersal ability relative to non-endemics, in the case of insects being weak or reluctant fliers, or flightless (Williamson, 1981; Wagner and Liebherr, 1992; Cox and Moore, 1993; Grant, 1998c; Bilton *et al.*, in press). Island endemic species may also be endemic *as a result of* poor dispersal ability (Ricklefs and Cox, 1972; Kunin and Gaston, 1993; Whittaker, 1998).

The second potential difference between endemics and non-endemics is in habitat availability. There may be less suitable habitat for endemics than is available for non-endemics, if the non-endemics are typically generalist species with high colonising ability and persistence (Barrett, 1998; Maurer, 1999). However, if endemics are adapted to a frequently occurring habitat, such as *laurisilva* streams, more habitats may be available for colonisation.

Finally, endemic and non-endemic species may differ in niche width. Endemic species may be more specialist, having narrower niches than non-endemics, because their longer evolutionary history on the islands, isolation and population bottlenecks afford the opportunity to evolve adaptations to specific local conditions (Grant, 1998c; Whittaker, 1998). This may lead to adaptive radiations (Orr and Smith, 1998; Schluter, 2000), which

have occurred within the Macaronesian fauna, although there is not much evidence for this in the freshwater fauna (Enghoff and Báez, 1993; Juan *et al.*, 2000). Further, endemics may have become increasingly specialist through lack of pressure on resources (until limited by intra-specific competition). Conversely, endemics may not be necessarily more specialist than non-endemics as oceanic islands can provide an opportunity for species to develop a wider niche width. This is because oceanic island communities may be unsaturated (Begon *et al.*, 1996; Brown and Lomolino, 2000a) allowing for density compensation (Cody and Diamond, 1975; Hildrew *et al.*, 1984) and, indeed, the absence of certain taxa in the Macaronesian freshwater fauna suggests that these communities are unsaturated (Stauder, 1991). Communities may also be unsaturated if local species richness is dependent upon regional species richness (Caswell and Cohen, 1993; Hugueny and Cornell, 2000) - as was found for these streams (Section 3.3.1). Thus, rather than evolving increased specialisation through adaptive radiation, endemic species may have become more generalist, taking advantage of this vacant niche space and of release from inter-specific competition (Malmqvist *et al.*, 1992; Grant, 1998c).

The set of species (endemics/non-endemics) with lower dispersal ability are predicted to have lower occupancy, as fewer streams would have been colonised (under the metapopulation and dispersal models). Likewise, the set of species with lower habitat availability is predicted to have lower occupancy, as fewer streams would provide suitable conditions (under the habitat availability model). Note that the effects of dispersal ability and habitat availability cannot be distinguished without additional evidence. Niche breadth acts primarily on the abundance of a species at a site (Tokeshi, 1993), as those with narrower niches will be more limited by resources than species with wider niches, and so have lower abundance (under the niche breadth model). Thus, whilst within the sets of endemic and non-endemic species an occupancy-abundance correlation is predicted, the

position of the data points (individual species) relative to the axes of occupancy and abundance may be different. If endemic and non-endemic species differ in occupancy and abundance, then any correlation between the two may be masked when analysing the total fauna; however within each set a significant correlation is expected (Figure 4.2). Any significant differences in the occupancy and abundance of endemic and non-endemic species will be used to infer qualitative differences in the above attributes (dispersal ability/habitat availability and niche breadth).

4.2 Methods

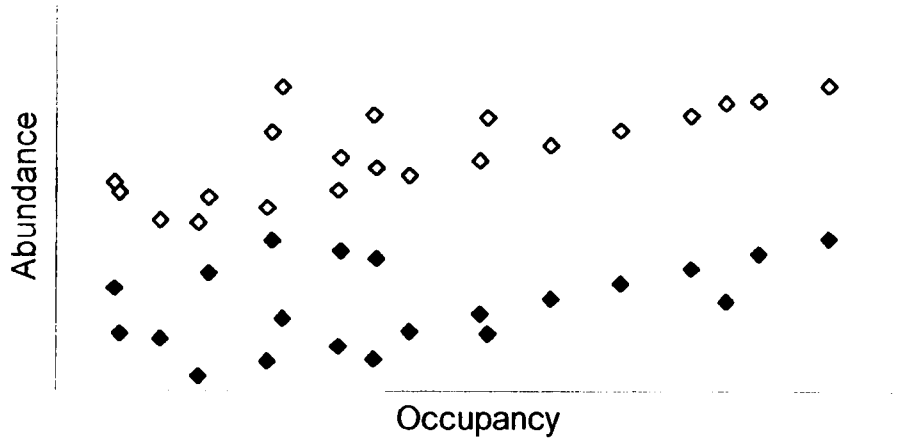
4.2.1 Parsimony analysis of endemicity

A species presence/absence matrix across islands was generated from all the sampling methods employed (Chapter 3) and supplemented by additional records for those species from the literature (Taxonomic Bibliography). Tenerife was split into two areas of separate origin: Anaga to the northeast, and the south and west of the island (Chapter 1). PAE cladograms of the islands were generated using PAUP 3.1 (Swofford, 1993). The cladogram was rooted with an outgroup being a hypothetical island with no species present (Rosen, 1988). A heuristic search with ten replicates of random step-wise addition was performed to find the most parsimonious cladogram (Felsenstein, 1985; Rundle *et al.*, 2000). MACCLADE 3.04 (Maddison and Maddison, 1992) was used to map the presence of individual species onto the final cladogram, and lists compiled of discriminating taxa at each node.

A.

Species sets differ in occupancy but not in abundance, as expected if they have differing dispersal ability or habitat availability, but the same niche width.

B.



Species sets differ in abundance but not in occupancy, as expected if they have differing niche width, but the same dispersal ability and habitat availability.

Figure 4.2 Potential occupancy-abundance relationships in two sets of species (*e.g.* endemics and non-endemics). Open and closed symbols represent species of two sets differing in dispersal ability or habitat availability (A) or in niche width (B). Note that a significant positive correlation is expected when the two species sets are treated separately but no correlation is expected when they are combined.

4.2.2 Nestedness

Species presence/absence data were analysed to test for nested species distributions with the Nestedness Temperature Calculator programme (Atmar and Patterson, 1993, 1995; Wright *et al.*, 1998). Matrices were produced for the Canary Islands, Madeira and Macaronesia, for the total species set, Coleoptera and Trichoptera (*i.e.* the most speciose orders). In the nestedness analysis procedure, the matrices are rearranged so that species distributions are maximally nested, that is, the taxa present at progressively more species-poor sites are subsets of the taxa present at all of the more species-rich sites. Deviations from perfect nestedness arise when species-poor sites have taxa not present at richer sites. An analogy with entropy is invoked. The matrix temperature *T*, between 0°C and 100°C, is a measure of the degree of departure from perfect nestedness (0°C is perfect order), taking into account both unexpected presence and unexpected absence. The 'fill' of the matrix is the percentage of cells where a species presence is recorded. Sites are ranked in terms of the number of species they support, with the richest in the top row of the matrix and the poorest at the bottom. Species are ranked from left to right, from those found at many sites to those found at only one. Idiosyncratic species are those whose distributions disrupt the overall pattern of nestedness, to a much greater extent than simply contributing to random noise, identified by their high temperature (greater than twice the mean). Idiosyncratic sites are those that similarly depart from nestedness, a consequence of the distribution of idiosyncratic species.

4.2.3 Trends in species richness with endemicity

Species presence/absence data were compiled for all sites and species were categorised as endemic (known only from Macaronesia) or non-endemic. The ratio of endemic to non-endemic species on the four islands and in the three land use types (*laurisilva*, pine forest and deforested land) was calculated and a Chi squared test used to

investigate whether the ratio of endemics to non-endemics differed between islands and land use types. Note that sampling effort was standardised across streams and though, the number of streams surveyed on each island differed, this is due to sampling all the available permanent streams on the Canary Islands therefore does not represent a sampling effect in terms of the recorded *island* richness total. The test was performed for the total data and Coleoptera (*i.e.* the only order with enough species to ensure an expected value of greater than or equal to five in each cell). The ratio of endemics to non-endemics was related to island biogeographical variables (isolation, area, altitude and age) using Pearson's product moment correlation co-efficient.

4.2.4 Occupancy, abundance and endemism

Occupancy-abundance relationships were investigated using data for Coleoptera, Ephemeroptera, Hemiptera, Mollusca, Odonata and Trichoptera. Occupancy was defined as the number of streams in which a species was present (from all the sampling methods employed) as a proportion of the number of streams surveyed in which it potentially could have been found. Canarian endemic species were assumed to occur potentially in a maximum of 31 streams (the number surveyed on the Canary Islands), whilst a Madeiran endemic could have been found in a maximum of 11 streams. That is, the Canary Islands were treated as a single biogeographic unit and all Canarian taxa were treated as if they could occur on any of the islands. Non-endemic species that were found on one archipelago were treated similarly, whilst the occupancy of species found on both the Canaries and Madeira was given as a proportion of the total number of streams sampled (42). The abundance of each species was summed for pool and riffle samples, and the median calculated across all the streams in which a species was recorded (Brown, 1995; Holt *et al.*, 1997). Note that this measure of abundance is actually a density estimate rather than a simple census: the sampling effort was fixed so species abundance recorded

depended upon density. The theoretical models discussed above (Section 4.1.5) apply equally to density and abundance. Those taxa not identified to a level at which their endemism status could be determined were omitted from this analysis.

A Wilcoxon signed ranks test was used to check for significant differences in occupancy and abundance between endemic and non-endemic species. Correlations between occupancy and abundance were investigated using Spearman's rank correlation co-efficient. The analysis was repeated with Coleoptera alone (the only order containing a sufficient number of both endemic (14) and non-endemic (20) species) as the models to be tested apply best to sets of 'ecologically similar' species (Section 4.1.5), and the Coleoptera are probably a more homogeneous group than the total macroinvertebrate stream fauna.

4.3 Results

4.3.1 Parsimony analysis of endemicity

One cladogram was retained, with a consistency index of 0.891; it was the shortest tree, of length 92 units. The cladogram and the discriminating species at each node are presented in Figure 4.3 and Table 4.2. Of the 18 species found on either the Canaries or Madeira, but not both, ten were endemic. However, within the Canaries only one quarter of the species discriminating La Palma from La Gomera-Tenerife were endemic, and no endemic species discriminated La Gomera and Tenerife.

4.3.2 Nestedness

Presence-absence matrices for Macaronesian, Canarian and Madeiran stream faunas were all significantly nested, having a temperature significantly lower than that

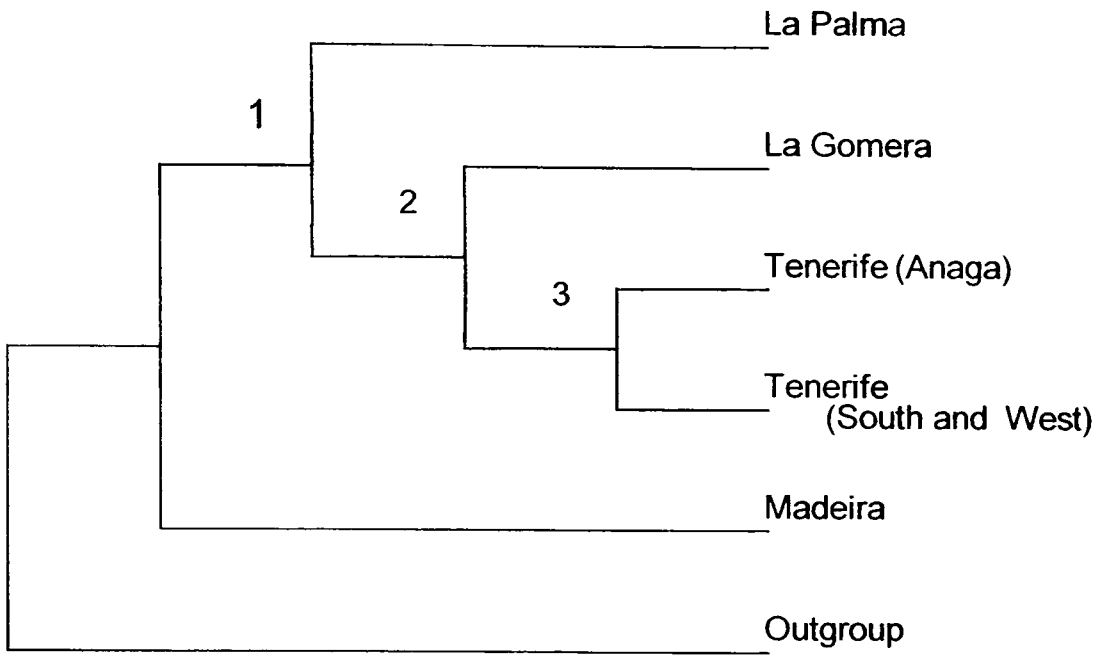


Figure 4.3 Cladogram of four Macaronesian islands based upon their stream macroinvertebrate faunal relationships, derived from PAE. Consistency index = 0.891, tree length = 92. Nodes are numbered as in Table 4.2. Note that this cladogram is identical to model C, Figure 4.1.

Node 1: shared by Canary Islands only		**Node 2: shared by La Gomera and Tenerife only**		**Node 3: shared by Tenerife regions only**
Agabus biguttatus		*Gyrinus dejeani*		*Bidessus minutissimus*
Anacaena haemorrhoa	E	*Gyrinus urinator*		*Coelostoma hispanicum*
Chaetarthria similis		*Haliplus lineaticollis*		*Helochares lividus*
Dryops gracilis		*Laccophilus hyalinus*		*Hydrochus grandicollis*
Hydraena serricollis	E	*Hydropsyche* sp.		*Hydroporus lucasi*
Hydroporus discretus		*Oecetis* sp.	E	*Caenis luctuosa*
Laccobius canariensis	E	*Oxyethira* spp.		*Pseudosuccinea columella*
Limnebius gracilipes		*Wormaldia taganananа*	E	*Crocothemis erythraea*
Nebrioporus canariensis	E			*Trithemis arteriosa*
Baetis canariensis	E			*Zygonax torrida*
Baetis pseudo./nigrescens	E			*Orthotrichia* spp.
Hydrometra stagnorum				
Notonecta canariensis	E			
Velia lindbergi	E			
Ancylus striatus	E			
Orthetrum chrysostigma				
Mesophylax aspersus				
Tinodes canariensis	E			

Table 4.2 Discriminating species at the nodes of the PAE cladogram of Macaronesian island faunal relationships. E: indicates Macaronesian endemic species.

expected from random permutations of the matrices ($p < 0.001$) (Table 4.3), but with a proportion of idiosyncratic species (Table 4.4). Within the Coleoptera and Trichoptera, nestedness was also significant ($p < 0.001$). The low fill of the matrices reflects the fact that few sites contain all species, and few species occur at all sites - many species having restricted distributions and sites tending to have only a small proportion of the species pool. Idiosyncratic species in the Macaronesian data set may reflect a disjunction in the faunas of the Canary Islands and Madeira (comprising more Madeiran species than were found to be idiosyncratic on Madeira alone).

4.3.3 Trends in species richness with endemicity

The four islands differed significantly in their numbers of endemic and non-endemic species (Chi squared test, all species: $p < 0.005$; Coleoptera: $p < 0.001$). (Table 4.5; Figure 4.4). La Palma and La Gomera had as many endemic as non-endemic species (ratio $\approx$ 1), however Tenerife had 68% more non-endemics than endemics (ratio = 0.59) and Madeira had nearly three times as many endemics as non-endemics (ratio = 2.67). The value of the ratio of endemics to endemics was not significantly correlated with any of the following island characteristics: isolation (distance from the African continent), area, altitude (a surrogate for habitat diversity) or island age (Table 4.6). However, the island with the greatest proportion of endemics, Madeira, was the most isolated island (Figure 4.5).

There were also differences between endemic and non-endemic species in their richness trends with land use (Table 4.7; Figure 4.6). The observed data differed significantly from a null hypothesis of constant numbers of endemic and non-endemic species across land use types (Chi squared test, all species: $p < 0.002$; Coleoptera: $p < 0.035$). Streams flowing through *laurisilva* had *circa* 50% more endemic species than those

Data Set	Matrix Size Species x Sites	Fill	Temperature °C	Idiosyncratic Species	Idiosyncratic Sites
Canary Islands	65x31	26.3%	17.62	10	4
Madeira	21x11	38%	21.21	2	1
Macaronesia	83x42	18.3%	20.37	11	5
Coleoptera	33x42	19.2%	18.17	4	3
Trichoptera	19x42	20.6%	22.58	1	4

Table 4.3 Nestedness of the Macaronesian stream macroinvertebrate fauna. See Section 4.2.2 for definitions of fill, temperature and idiosyncrasy terms.

Canary Islands	Madeira	Macaronesia
Ancylus striatus (Ancylidae)	*Ancylus fluviatilis* (Ancylidae)	*Ancylus striatus* (Ancylidae)
Agabus biguttatus (Dytiscidae)	*Stactobia* spp. (Hydroptilidae)	*Velia lindbergi* (Veliidae)
Velia lindbergi (Veliidae)		*Hydroporus discretus* (Dytiscidae)
Hydraena serricollis (Hydraenidae)		*Anacaena haemorrhoa* (Hydrophilidae)
Hydroporus discretus (Dytiscidae)		*Tinodes canariensis* (Psychomyidae)
Anacaena haemorrhoa (Hydrophilidae)		*Baetis rhodani* (Baetidae)
Microvelia gracillima (Veliidae)		*Hydropsyche maderensis* (Hydropsychidae)
Tinodes canariensis (Psychomyidae)		*Wormaldia taganananа* (Philopotamidae)
Agapetus adejensis (Glossosomatidae)		*Stactobia* spp. (Hydroptilidae)
Agabus nebulosus (Dytiscidae)		*Ancylus fluviatilis* (Ancylidae)
		Agabus nebulosus (Dytiscidae)

Table 4.4 Macaronesian stream macroinvertebrate taxa that did not conform to a pattern of nested distributions. Idiosyncratic taxa (Section 4.2.2) in three data sets are listed in order of occupancy, from highest to lowest.

Group	La Palma		La Gomera		Tenerife		Madeira	
No. of streams	12		10		9		11	
	E	N	E	N	E	N	E	N
Coleoptera	5	7	8	7	7	18	5	1
Amphipoda	0	0	1	0	0	0	0	0
Ephemeroptera	2	1	2	1	2	2	1	1
Hemiptera	1	2	1	3	2	3	1	0
Mollusca	1	0	1	3	1	4	0	3
Odonata	0	0	1	1	1	7	1	0
Trichoptera	3	2	5	3	9	3	8	1
Total richness	12	12	19	18	22	37	16	6
Ratio E:N	1		1.06		0.59		2.67	
% Total pool	31	28	49	42	56	86	41	14

Table 4.5 Variation in richness of endemic and non-endemic Macaronesian stream macroinvertebrates with island. Records obtained from the present study only. E: endemic; N: non-endemic. The percentage of the total endemic and non-endemic Macaronesian species pool occurring on each island is also shown.

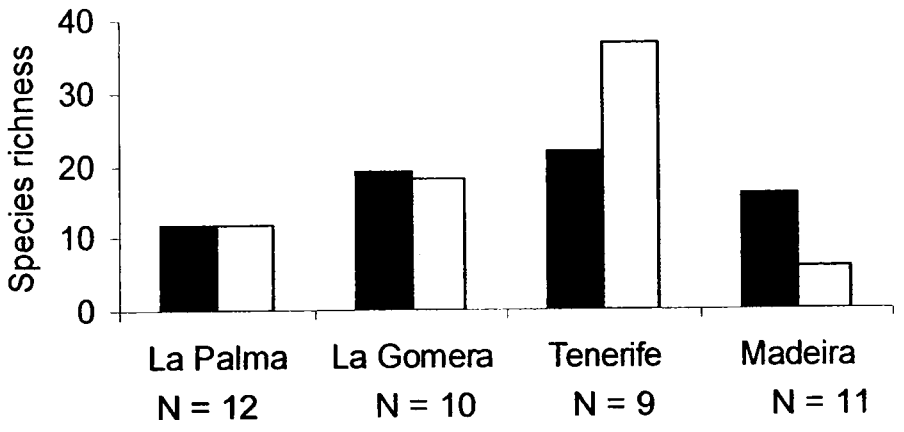


Figure 4.4 Variation in total richness of endemic and non-endemic Macaronesian stream macroinvertebrates with island. Endemic species indicated in black, non-endemics in white. Species richness for the Canary Islands is a total (given the sampling method used) as *all* permanent streams on the islands were sampled. Species richness for Madeira was estimated for comparison by sampling a similar number of streams. N: number of streams surveyed.

Factor	*r*	*p*
Isolation	0.790	0.210
Area	-0.175	0.825
Altitude	-0.345	0.655
Age	0.421	0.579

Table 4.6 Correlation between the ratio of endemic to non-endemic stream macroinvertebrate species and island characteristics of four Macaronesian islands. See Chapter 1 for data sources. Pearson's product-moment correlation co-efficient and *p* value of the linear regression model are given.

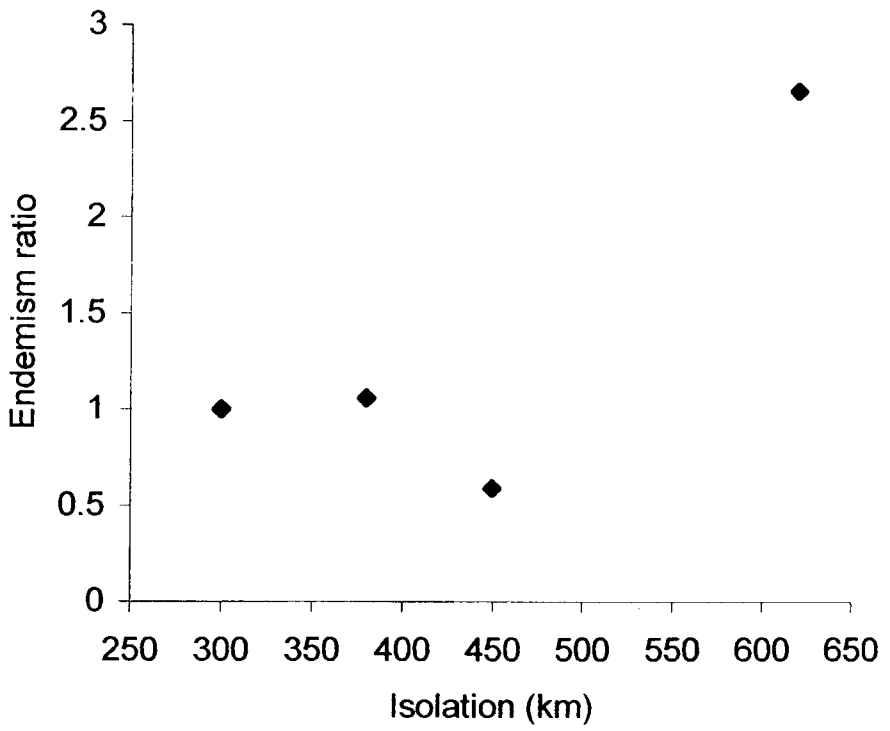


Figure 4.5 Relationship between the ratio of endemic to non-endemic stream macroinvertebrate species and isolation of four Macaronesian islands. Isolation is given in terms of distance from the African continent (Morocco).

Group	Laurisilva		Pine		Deforested	
No. of streams	26		10		6	
	E	N	E	N	E	N
Coleoptera	14	8	6	15	7	15
Amphipoda	1	0	0	0	0	0
Ephemeroptera	3	2	2	2	3	3
Hemiptera	2	2	2	2	2	4
Mollusca	1	5	1	3	1	5
Odonata	1	0	1	3	1	7
Trichoptera	13	3	6	3	9	3
Total richness	35	20	18	28	23	37
Ratio E:N	1.75		0.64		0.62	
% Total pool	90	47	46	65	59	86

Table 4.7 Variation in richness of endemic and non-endemic Macaronesian stream macroinvertebrates with catchment land use. Records obtained from the present study only. E: endemic; N: non-endemic. The percentage of the total endemic and non-endemic Macaronesian species pool occurring in each land use type is also shown.

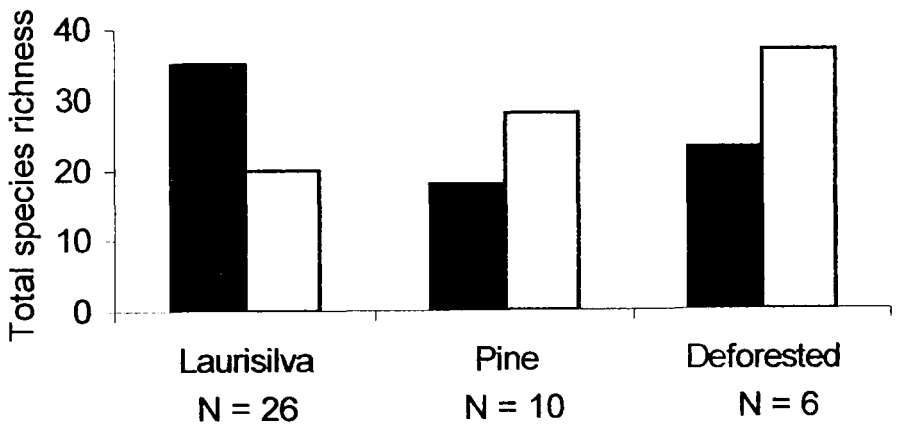


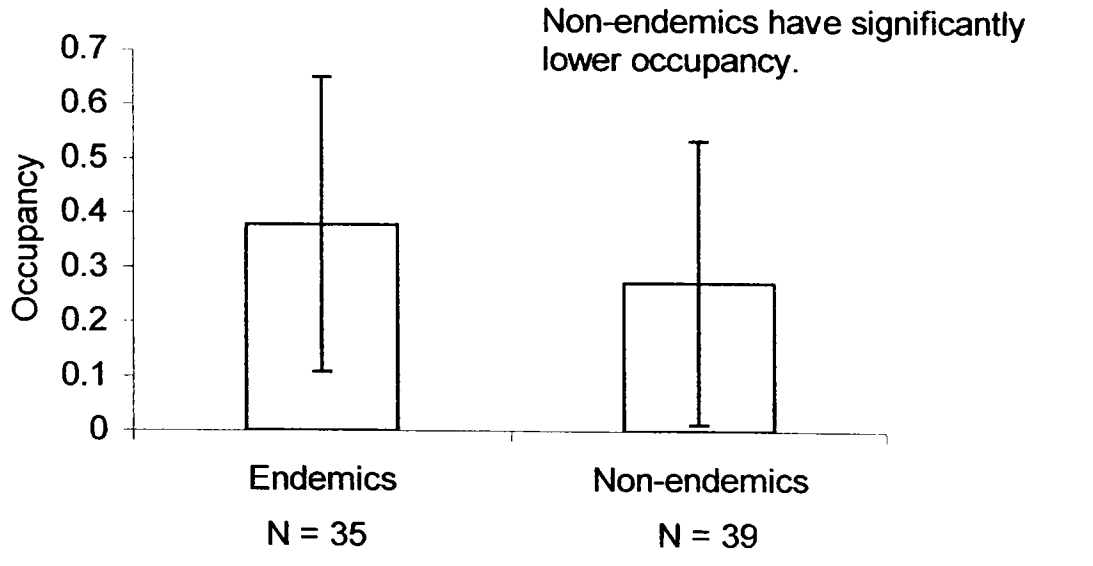
Figure 4.6 Variation in total richness of endemic and non-endemic Macaronesian stream invertebrates with catchment land use. Endemic species indicated in black, non-endemics in white. Species richness for the Canary Islands is a total for all streams in each land use type (given the sampling method used). All permanent pine forest streams were sampled. All *laurisilva* and deforested permanent streams on the Canary Islands were sampled; species richness for Madeira was estimated for comparison by sampling a similar number of streams. N: number of streams surveyed.

of other land use types (35 species, compared to 18 and 23), and *circa* 60% fewer non-endemics (20 species, compared to 28 and 37). The ratio of endemics to non-endemics was therefore much higher in *laurisilva* streams (1.75) than in pine forest or deforested streams (0.64 and 0.62 respectively), with 90% of the total endemic species pool occurring in *laurisilva* streams (Table 4.7). Whilst the number of streams occurring in each land use type varied, the total species richness did *not* increase with number of streams sampled (Section 3.3.1), therefore the high richness of endemics observed in *laurisilva* streams is not an artefact of uneven sample sizes.

4.3.4 Occupancy, abundance and endemism

Seventy-four species were included in the analysis, of which 47% (35 species) are endemic to Macaronesia. Endemic species occupied significantly more streams than non-endemics (Wilcoxon signed ranks test, $p < 0.024$) (Figure 4.7). However, endemics and non-endemics did not differ significantly in abundance (Figure 4.7). No significant differences in occupancy or abundance were found between endemic and non-endemic Coleoptera.

Endemic species did not show a significant relationship between occupancy and abundance even when outliers (*i.e.* species occurring in high abundance at single sites), *Lepidostoma tenerifensis* (Trichoptera: Sericostomatidae) and *Chaetogammarus chaetocerus* (Amphipoda: Gammaridae), were excluded. However, non-endemics exhibited a significant positive relationship between occupancy and abundance ($p < 0.005$, correlation co-efficient = 0.438, $R^2 = 19.2\%$) (Figure 4.8). For Coleoptera this pattern was reversed: endemics showed a stronger occupancy-abundance relationship ($p < 0.010$, correlation co-efficient = 0.736, $R^2 = 54.2\%$), whilst the non-endemic species did not show



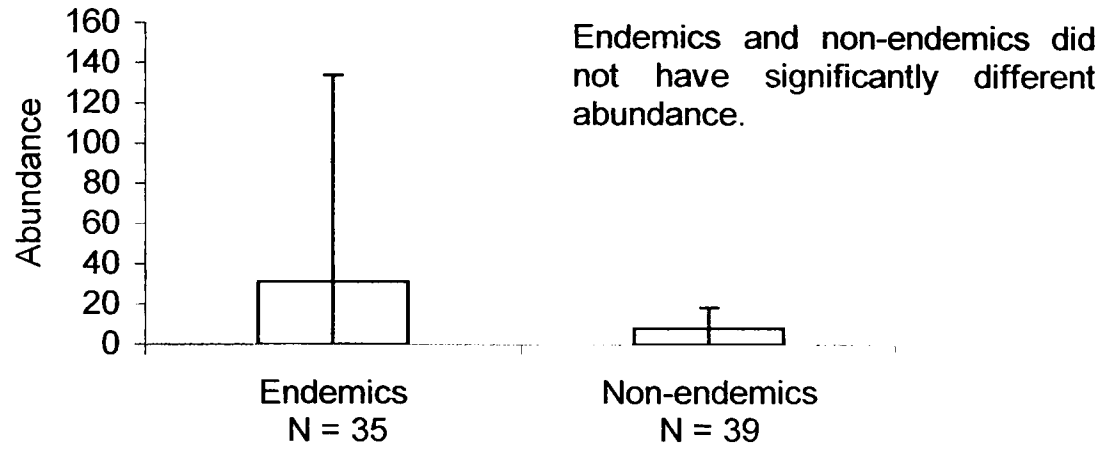
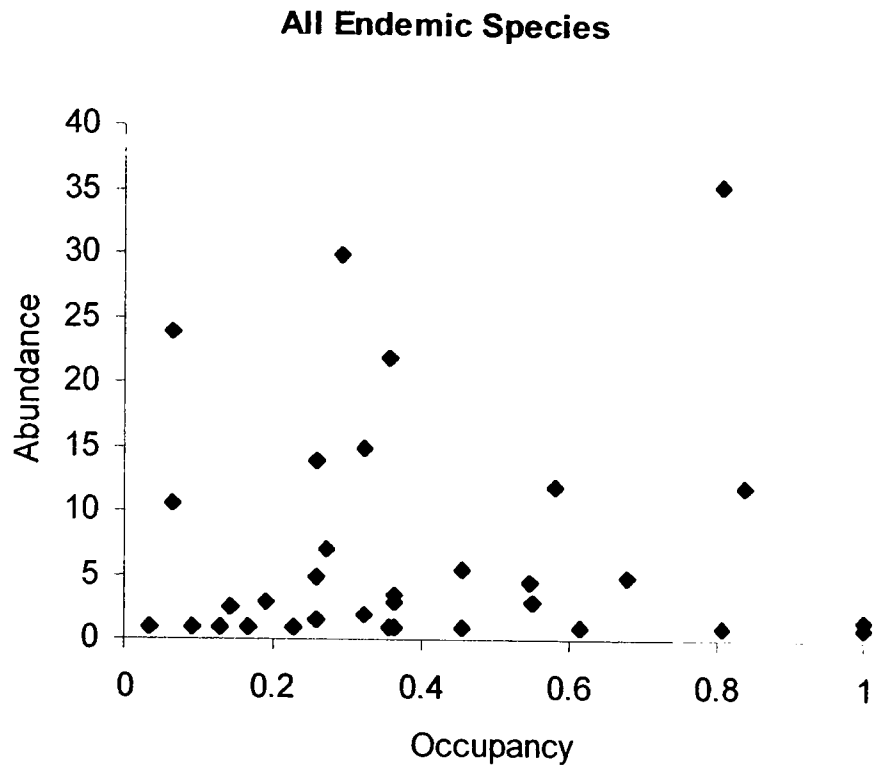


Figure 4.7 Occupancy and abundance of Macaronesian endemic and non-endemic stream macroinvertebrates. Standard deviation is shown. N is number of species.



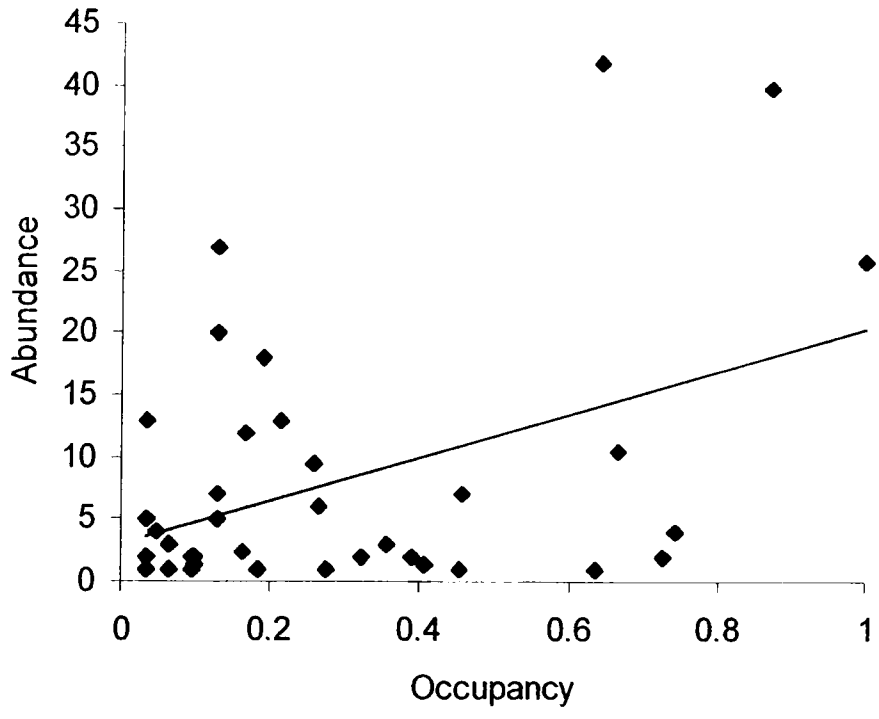
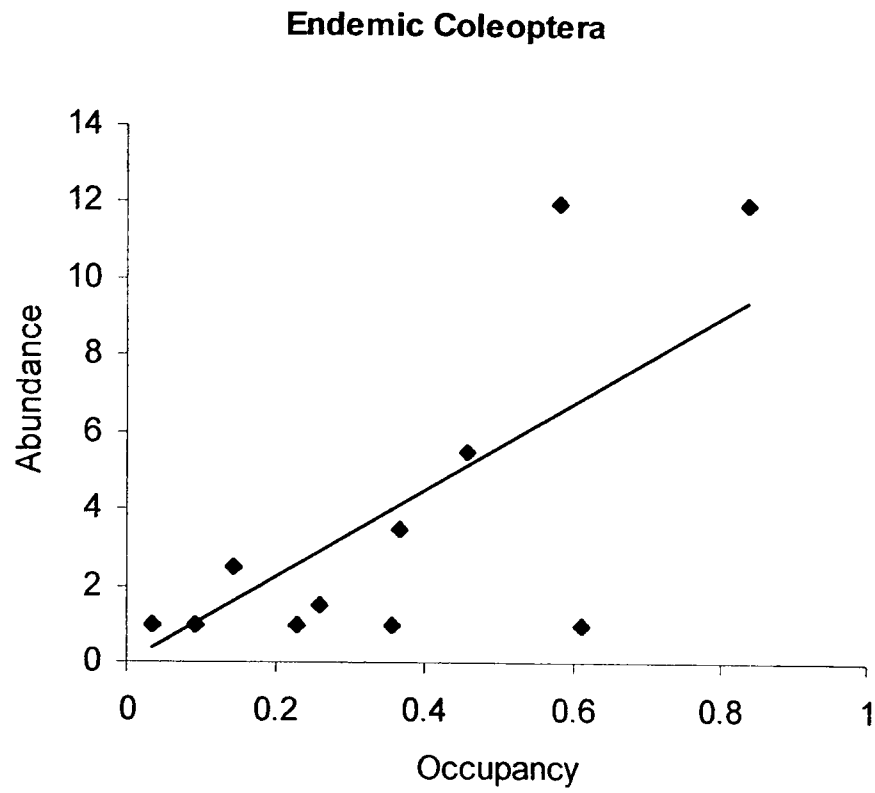


Figure 4.8 Occupancy plotted against abundance for endemic and non-endemic Macaronesian stream macroinvertebrates. Simple linear regression line is shown where significant (see text). Outliers excluded. Note that the above plots are an example of the pattern more clearly displayed in Figure 4.2 (A) — the points for endemic species are, on average, displaced to the right (higher occupancy). For clarity, the two sets of species are not displayed on the same plot in this case due to the number of superimposed points.



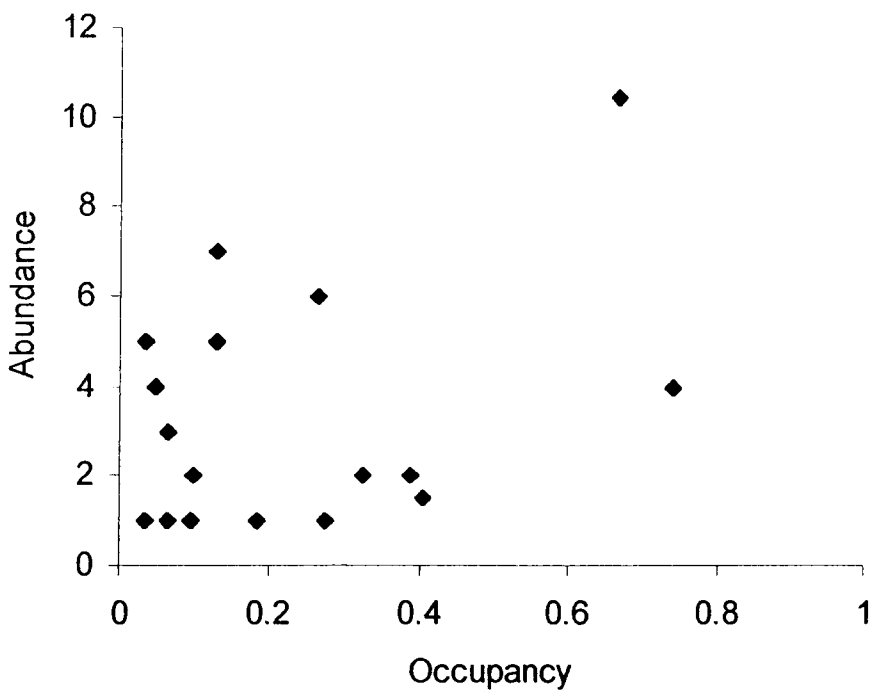


Figure 4.9 Occupancy plotted against abundance for endemic and non-endemic Macaronesian aquatic Coleoptera. Simple linear regression line is shown where significant (see text). Note that the points for endemic Coleoptera are not displaced relative to those for non-endemic Coleoptera. For clarity, the two sets of species are not displayed on the same plot in this case.

a significant relationship (Figure 4.9). No relationship was found for endemics and non-endemics combined.

4.4 Discussion

4.4.1 Parsimony analysis of endemicity

PAE was used to elucidate faunal relationships between the four islands studied. The results of PAE were consistent with those of Malmqvist *et al.* (1997) for Ostracoda: they found that Madeira grouped separately from the Canary Islands and, within the Canaries, La Palma grouped separately from La Gomera and Tenerife. This grouping reflects the geographical proximity of the islands (Figure 4.1, model C), and contrasts with the community analysis (Chapter 3) in which the faunas of La Palma and Tenerife were not significantly different. This is due to the absence of the less common species on La Palma, but otherwise broad similarity in the dominant species. Distance-dependent inter-island dispersal and colonisation account for this pattern of decreasing faunal similarity with increasing distance.

The roles of endemic and non-endemic species in the faunal relationships between the islands are different. On the Canary Islands, endemic species tend to be widespread across the islands (concordant with the higher occupancy found for endemics) whilst many non-endemics occur only on Tenerife. Endemic species thus are important in discriminating between the archipelagos (Canary Islands and Madeira), whilst, within the Canaries, island relationships are determined by their non-endemic fauna. It would be of interest to repeat the analysis with similarly-gathered data on the species present in Iberian and Moroccan streams.

This cladistic analysis is at the largest scale within the scope of this study and illustrates the island species pool relationships. It is within the constraints of these species pools that smaller scale processes such as the interaction of the fauna with physicochemical environment take place (Schluter and Ricklefs, 1993). One of these processes is dispersal and colonisation: the following chapters use genetic analysis to infer the degree to which assemblages exchange individuals of selected species with one another at the inter- and intra-island scales.

4.4.2 Nestedness

The significant nestedness found indicates that the most species-rich sites contain the majority of taxa, with the species-poor sites containing decreasing subsets of the fauna such that the most depauperate sites contain only the ubiquitous species. Hanski's 'core' species (Hanski, 1982a, b, c) are those that are constant throughout the subset of a nested biota (Patterson, 1990). The significant nestedness is an indication of low beta diversity (Wright and Reeves, 1992), that is, homogeneity of the stream fauna (presence/absence data) at the regional and archipelago level. In this system, nestedness may be caused by extinction at depauperate sites, but may also be due to a combination of inter-site variation in environmental conditions, variation in the niche requirements of species and the stochastic and deterministic components of dispersal (Wright *et al.*, 1998). Variation in species richness with stream physicochemistry has been demonstrated (Section 3.3.3); only a small subset of the species pool was found in streams poor in ions and with high pH. The ubiquitous species may be those that can utilise the widest range of habitats (large niche width and high habitat availability), the best dispersers/colonisers, or may be the most locally abundant (having wide distributions as a result of passive sampling). The distributions of more specialist species are nested within those of more generalist species, that is, ecological range is likely to be correlated with geographical range size (Law, 1999).

Indeed, nested patterns of habitat utilisation necessarily imply discontinuities in ecological range distributions (Kolasa, 1996).

Idiosyncratic species are also a prominent feature of this analysis. Idiosyncratic species whose distributions are predominantly influenced by some process other than that giving rise to the nestedness. For example, the distribution of new immigrants may be biased towards a subset of sites; there may be a fundamental disjunction in the historical evolution of community structure; and competitive exclusion could give rise to distinctly idiosyncratic distributions (Atmar and Patterson, 1993; Wright *et al.*, 1998). Malmqvist and Hoffsten (2000) suggested that deviations from nestedness in Swedish stream fauna could be due to taxa being sensitive to particular biotic interactions or restricted to certain environmental conditions. Fugitive/'supertramp' species (Diamond, 1975), having a strategy of good dispersal ability but low competitive ability, will tend to depart strongly from nestedness because they are usually found only at sites/islands with low species richness (Whittaker, 1998).

In the present study, the two species of *Ancylus* may behave as 'supertramp' species as, though passively dispersed between streams, their flexible breeding system may increase the chances of dispersal leading to successful colonisation and establishment (Section 8.4.5), though not in the most species-rich streams. Other species may have similar 'strategies', for example, *Anacaena haemorrhoa* (Coleoptera: Hydrophilidae) and *Agabus nebulosus* (Coleoptera: Dytiscidae), often occur in open, disturbed or newly created habitats. Some idiosyncratic species were habitat specialists, for example, *Microvelia gracillima* (Hemiptera: Veliidae) occurs in small shaded streams, and *Agabus biguttatus* (Coleoptera: Dytiscidae) in cold, high altitude streams (d'Orchymont, 1940; Balke *et al.*, 1990; Malmqvist *et al.*, 1993). In other cases, idiosyncratic distributions may

have arisen due to the difference between the fundamental and realized niche, in species that are poor competitors or poor dispersers. Endemism on the Canary Islands and Madeira will also account for much of the idiosyncrasy in species distributions (*e.g. Baetis rhodani* (Ephemeroptera: Baetidae) and *Wormaldia tagananana* (Trichoptera: Philopotamidae)), breaking the assumption that the species in the study system have shared biogeographic histories. The 'separateness' of the fauna of Madeira implies that it is not part of the same regional species pool as the Canaries and does not experience the same level of inter-island dispersal, for example.

The presence of idiosyncratic species means that they might be omitted from conservation measures directed towards the most species-rich sites, whilst the nestedness pattern may demonstrate the likely order of extinction of species - those limited to a few species-rich sites are most vulnerable (Malmqvist *et al.*, 1997). The physicochemistry of streams with respect to species richness was discussed in Section 3.4.3. The most species-rich sites were those with low pH, and high conductivity, magnesium and hardness (Table 3.7), but occurred in a variety of land use types (Appendix 3.2).

4.4.3 Trends in species richness with endemicity

In contrast to the null hypothesis of a constant ration of endemics to non-endemics across the islands, the most isolated island, Madeira, had a greater proportion of endemic species in its fauna than the other islands. This is to be expected as geographic isolation decreases the probability of continental species colonising, and the lack of gene flow once a species does colonise increases the likelihood of the population evolving into a new, distinct endemic species (Enghoff and Báez, 1993; Grant, 1998c; Whittaker, 1998).

The second null hypothesis, that richness of both endemics and non-endemics would be constant across land use types, was also refuted. Endemic and non-endemic species did not occur randomly with respect to land use, *laurisilva* streams having a significantly higher number of endemic species than pine forest or deforested streams. Overall, a greater proportion of the endemic species pool occurred in *laurisilva* streams than in other land use types, and more of the non-endemic species pool occurred in deforested streams than other stream types. Finally, *laurisilva* streams, as a group, also contained more endemic than non-endemic species, and the converse was true for deforested streams. Thus, all three land use types had distinct faunas. This differs from the results in Chapter 3, as the analyses in the present chapter used presence/absence data (equally weighting all species, however rare) rather than characterising streams by the most abundant and constant species.

Factors that influence species richness include biotic interactions (*e.g.* competition, predation, mutualism) population density (itself dependent on behaviour, physiology, survival rate, reproductive rate, immigration and emigration) and resource availability. These factors are hierarchical, from the level of individual properties (behaviour and physiology, determining niche width) through population dynamic factors and community diversity to ecosystem properties (resource availability) (Maurer, 1999). Of these factors, habitat availability is likely to differ for endemics and non-endemics due to different adaptations: the concentration of endemic species in *laurisilva* streams may in part be due to the presence of relictual endemics that are specialists of that habitat and not adapted to other habitats. However, in addition to habitat specialism, historical factors may play an important role in producing the observed endemic species richness patterns, for example if endemics are particularly vulnerable to local extinction in sites disturbed by mankind's activities. Finally, the more-species rich, and potentially more stable, communities in

laurisilva may be resistant to invasion by more recently arrived non-endemic species (Begon *et al.*, 1996; Law, 1999), producing differentiated communities dominated by endemics and non-endemics.

As the Macaronesian stream fauna contains so many species of restricted range, the geographic distribution of species richness is an important consideration for conservation planning (Allen and Flecker, 1993; Malmqvist *et al.*, 1993; Malmqvist and Hoffsten, 2000). If all the streams were to take on the character of deforested streams, 41% of endemic species may be lost. In contrast, only 10% of endemic species were *not* recorded in *laurisilva* streams. Malmqvist *et al.* (1993) emphasised the individuality of the streams on Tenerife and the need to preserve as many of them as possible to protect the full diversity of the fauna. Kinzig and Harte (2000) discuss the conservation implications of the spatial distribution of endemics, and how high levels of endemism can make generalities such as the species-area relationship, often used in conservation planning, inappropriate.

4.4.4 Occupancy, abundance and endemism

Endemics and non-endemics did not differ in their abundance but endemic species had significantly greater occupancy than non-endemics. This was not clear from Figure 4.7, but the difference in occupancy between endemics was small yet consistent; however, both sets of species include outliers contributing to the large standard deviations (see also Figure 4.8). These results suggest that endemics and non-endemics do not differ in niche width and that endemics are better dispersers and/or have a greater amount of habitat available for them to exploit. The latter scenario is more likely as the majority of endemic species richness is concentrated in *laurisilva* streams, whilst non-endemic richness is greatest in deforested streams and there are far more *laurisilva* streams on the islands than any other type. In addition, it is usually the case that endemics are poorer dispersers than

non-endemics (Section 4.1.5), but their long history on the islands would have enabled them to exploit the available habitats.

The differences between endemic and non-endemic species can be used to test the relative influences of the different mechanisms that may produce occupancy and abundance patterns. As well as discounting potential niche width differences, the fact that there was no difference in abundance between endemics and non-endemics suggests that the geographic range structure model (Maurer, 1999) is not appropriate in this case (Gaston *et al.*, 1997a). Some species have low occupancy because their geographic range is so small (the endemics) and others (potentially) because they are on the edge of their range (the non-endemics). These 'peripheral' species would tend to have low abundance under the geographic range structure model whilst those with small ranges are predicted to have a higher mean abundance as their optimal as well as marginal habitats have been sampled. Another model that can be rejected is that arising from taxon cycle (Ricklefs and Cox, 1972). This predicts that, at an early stage in the evolutionary process, species have high occupancy and abundance. Populations then fragment to produce endemic species with low occupancy and abundance. Evidence for this was found in the terrestrial Hemiptera of the Canaries (Sergel and Báez, 1990) but the theory is not supported by the present data set.

Whilst the non-endemic species showed the expected positive correlation between occupancy and abundance, the endemics did not: endemic species included those with high occupancy and low abundance, and vice versa. The occupancy-abundance correlation may be one of the few ecological generalities, along with the species-area relationship to which it is logically connected (Hanski and Gyllenberg, 1993; Hanski *et al.*, 1993; Gaston *et al.*, 1997b). Exceptions to the rule, such as the Macaronesian endemic species, may prove particularly informative. Occupancy and abundance become decoupled (*e.g.* an increase in

one parameter does not automatically lead to an increase in the other) if there is little dispersal between sites. That is, high local abundance within a favoured habitat does not lead to high occupancy of habitats if there is no emigration, unless the circumstances producing high abundance occur at many sites, or if local extinction and recolonisation were not in equilibrium at the time of sampling. However, whilst the abundance and occupancy of individual species may vary over time, there is no reason to expect shifts in the emergent properties of the endemic and non-endemic species sets as a whole. Within the Coleoptera, the reverse patterns may have arisen by chance due to the small number of data points available. The power of the analysis is limited by the small size of the Macaronesian aquatic fauna when the set is subdivided (Blackburn *et al.*, 1990), and large data sets are usually required for this kind of analysis, *e.g.* Gotelli and Simberloff (1987).

As in the present study, documented inter-specific occupancy-abundance relationships are rarely strong: the median variance (R^2) explained by statistically significant analyses is 20-30% (Gaston, 1996b). Scatter around the regression line may be due to combining orders that differ in properties such as body size and dispersal ability or the effects of more than one mechanism, with different mechanisms affecting different taxa to different extent (Gaston and Curnutt, 1998). Quinn *et al.* (1997) found no life history or functional group variable that could explain significant variation around the regression line for Lepidoptera, and there are likely to be multiple interacting causes of the variation around the occupancy-abundance relationship. However there may be no detectable relationship between body size and density/abundance in many animal communities (Blackburn *et al.*, 1990). Whilst all the species are associated with small streams, the orders encompass a range of ecological attributes, and the streams have different physicochemical attributes. This brings an error into the occupancy term, as without

detailed autecological research it is impossible to know how many streams each particular species could actually occupy.

Within individual species, high occupancy and high abundance do not necessarily co-occur (Gaston and Curnutt, 1998), although this is not usually expected to over-ride the general occupancy-abundance relationship. Those exceptional species with high abundance and low occupancy (the endemics *Lepidostoma tenerifensis* and *Chaetogammarus chaetocerus*) are likely to have large effective population sizes otherwise the probability of extinction would be high. These species may be specialised to a resource that is only locally abundant, may have suffered local extinction at other sites (with high abundance due to a time lag in the relationship) or inhabit a very stable, long-lived environment (they both occur in *laurisilva*). In addition they may be the product of idiosyncratic evolutionary processes, for example *C. chaetocerus* is assumed to be descended from a marine ancestor that moved upstream into freshwater, an origin not shared with the other stream fauna. The highly clumped distribution typical of rare species (Hartley, 1998) is taken to an extreme in the above examples, contributing the lack of an occupancy-abundance relationship. For those with low abundance at a large proportion of sites (*e.g. Limnebius gracilipes* (Coleoptera: Hydrophilidae)) a significant degree of inter-site dispersal is expected, otherwise individual small populations would be vulnerable to extinction by stochastic processes. However, abundance may also be underestimated by the sampling method, for example *L. gracilipes* is a specialist of the stream margin (d'Orchymont, 1940), a habitat not specifically sampled in the present study.

Chapter 5

Allozyme Analysis in Freshwater Biology:

Studies of Evolution, Ecology and Biogeography

Allozyme Analysis in Freshwater Biology: Studies of Evolution, Ecology and Biogeography

SUMMARY

The study of protein variation in the form of allozymes remains a useful method of obtaining genetic information about an individual, population or species. The technique is relatively quick, cheap and easy, and detects genetic variation at a resolution that is appropriate for tackling a wide range of taxonomic, evolutionary and ecological problems. The general principles of allozyme electrophoresis are explained, including some of the advantages and limitations of the method, and the statistical techniques commonly employed to analyse allozyme data. The contribution of this approach to many aspects of freshwater invertebrate ecology, evolution and biogeography is reviewed for the first time. The review will focus on studies of population genetic structure and dispersal - those most relevant to the subjects of this thesis (*e.g.* Jackson and Resh, 1992; Colgan and Ponder, 1994; Dillon and Wethington, 1995; Jarne and Städler, 1995; Bunn and Hughes, 1997). Many of the applications of allozyme techniques have not yet been fully exploited. In particular, previous studies have often been compromised by a poor design, and only a minority of taxa has been examined. There remains potential for the use allozyme electrophoresis to investigate aspects of the evolution, ecology and biogeography of freshwater invertebrates. In subsequent chapters, allozyme studies to infer the extent of inter-population dispersal, both between and within islands, for selected species are presented. Allozyme data is used to test hypotheses about the effect of population history, environmental patchiness and dispersal ability and mechanism on genetic variation, population structure and gene flow.

5.1 Introduction

5.1.1 General principles

Allozyme analysis is the study by gel electrophoresis of protein variation due to underlying genetic sequence variation. This genetic variation may be either neutral (subject to changing demographic parameters) or adaptive (a product of natural or sexual selection) (Kimura, 1991). Allozymes are different forms of an enzyme produced by different alleles at a locus (Avise, 1975). (Isozyme is a general term for different enzyme forms, which may be allozymes or may be produced from different genes but have the same function). Many enzymes are genetically polymorphic and the detectable polymorphisms can be recorded as informational characters (Ayala, 1983). Electrophoresis distinguishes different proteins by their rate of migration through a gel under the influence of an electric field. The migration rate depends on the net electric charge, shape and size of the protein. Therefore, different migration distances reflect amino acid differences, which themselves reflect DNA sequence differences. Histochemical staining reveals the iso-electric focus (electrical equilibrium position) of the protein. Easteal and Boussy (1987) describe modifications to the technique, making it more suitable for studies on small invertebrates: using cellulose acetate sheets rather than starch or polyacrylamide gels. (Allozymes run on cellulose acetate gels are separated by differences of charge only, not molecular size).

An individual may be homozygous or heterozygous at each locus; allozyme frequencies in the population are informative. In addition, diploid genotype frequencies are used as information in mating system analysis. When a number of loci are studied, similarity or distance coefficients can be produced to compare populations or distinguish species (Avise, 1975). The differences that arise between populations are due to a

combination of mutation, genetic drift, selection and the pattern of gene flow. Whilst it may be possible to test for the influence of selective factors, this is not necessary for many of the applications of the technique, and neutral evolution alone is often sufficient to account for observed patterns of variation (Kimura, 1991).

5.1.2 Allozyme variation and underlying genetic variation

The relationship between allozyme variation and DNA variation is not direct. Allozyme variation consistently underestimates variation in the underlying genetic sequence for two reasons. Firstly, the redundance of the genetic code, where most amino acids are specified by more than one codon, means that a single nucleotide mutation, particularly when in the third position of the triplet, may not produce any change to the amino acid sequence. Secondly, allozymes are distinguished by their differing mobility on the gel, which reflects their different ionic charge, shape and size. Those with the same apparent mobility have the same net ionic charge on the amino acid side chains, but not necessarily identical amino acid sequences.

Intensive study of *Drosophila melanogaster* enzymes has shown that there are sometimes many more alleles than can be detected by one electrophoretic technique alone (Coyne, 1982). Sequential or two-dimensional electrophoresis may show that the bands visualised are caused by more than one allozyme. In general, it is the enzymes already known to be polymorphic that show this extra variation and so initial estimates of polymorphism remain relatively unchanged, whereas estimates of heterozygosity may increase dramatically (Lewontin, 1991; Hartl and Clark, 1997).

The indirect link between allozyme variation and underlying genetic variation does not preclude, however, the usefulness of gel electrophoresis. This is because allozyme studies are essentially comparative, rather than absolute determinations of genotypes.

5.1.3 Allozymes and the molecular clock

The molecular clock refers to the observation that the rate of molecular evolution can be uniform over long periods (Gillespie, 1991; Gaut, 1999). Differences in the number of nucleotide substitutions or amino acid replacements between certain molecules of pairs of organisms can be used to estimate their time of divergence. The molecular clock is calibrated, albeit approximately, with geological data, for example the date of formation of a physical feature which gave rise to allopatric speciation, with information from the fossil record or by using rates of molecular evolution previously calculated for related species. The constant rate of the molecular clock assumes a constant rate of neutral mutation, and likewise, statistical tests of genetic divergence such as Nei's genetic distance assume a constant rate of molecular evolution (Nei, 1972). The correlation of Nei's genetic distance with time has been calculated as roughly 14 million years for a distance of one unit (Maxson and Wilson, 1979). This was used by Pashley *et al.* (1985) to infer the sequence of island colonisation and speciation in the South Pacific *Aedes (Stegomyia) scutellaris* subgroup (Diptera: Culicidae).

However, it is generally inappropriate to apply the molecular clock to allozyme studies. Firstly, it may not be strictly correct to extrapolate from an electrophoretic study to describe the likely variation of the entire genome. For example, it is possible that those enzymes chosen for electrophoresis are more polymorphic than other, more highly substrate-specific enzymes. Secondly, allozyme electrophoresis provides no information

about the number of mutational changes that may have produced the observed variation and different enzymes are known to evolve at different rates, thus calibration of the molecular clock is difficult (Skibinski and Ward, 1982). The appearance of novel electromorphs is not necessarily linearly related to the underlying gene mutation. Finally, past population bottlenecks skew present allele frequency distributions, and the assumption of neutral evolution is unlikely to be met consistently as at least some enzyme systems are likely to be, or have been, under selection pressure (Gillespie, 1991; Kreitman and Akashi, 1995; Gaut, 1999). However, effective population sizes and mutation rates can only be estimated so in most cases the data are fitted to a model of neutrality.

5.1.4 Advantages

Some of the advantageous features of allozyme analysis are common to all molecular analysis methods: objectivity, unweighted characters, common function at a locus implies homology, and relative similarities can be calculated, even between widely divergent groups (Avise, 1975). The differences between conspecific populations revealed by allozyme analysis are generally small, with less than 15% of loci having non-identical allele distributions, but between even sibling species the differences are often much greater (Avise, 1975). Hence, a small number of individuals may be used to characterise a population and sympatric sibling species can be distinguished and arranged by their percentage of shared genotypes. Allozyme analysis is also a straightforward, cheap, quick and flexible technique that provides resolution over scales suitable for investigating a wide range of ecological and evolutionary questions (Lewontin, 1991), including the exploration of population differentiation and interpopulation dispersal of the present study. This has encouraged use of the technique and the proliferation of comparative data (*e.g.* Brown and Richardson, 1988; Jarne and Delay, 1991; Jarne and Städler, 1995).

Electrophoresis performed with cellulose acetate gels has additional advantages as it gives particularly good band resolution, and running and incubation times are short. The gels are bought pre-formed, saving further time and increasing repeatability of results. The method is also suitable for very small organisms, including meiofauna (Boileau *et al.*, 1992), as a minute quantity of substrate (< 1μl) is applied to the gel, allowing repeat screenings of individual samples. Easteal and Boussy (1987) demonstrated that cellulose acetate gels give results with a sensitivity equal to, or improving upon, starch and polyacrylamide gel electrophoresis and list additional advantages of this type of electrophoresis - improvements in terms of expense, convenience, and reduced health and safety risks.

5.1.5 Disadvantages

The potential problems of using allozyme analysis to measure gene flow or reconstruct phylogeny are mostly statistical. Avise (1975, 1983) drew attention to two areas of concern. Firstly, sampling error may be large. The frequency distribution of allozymes in the individuals sampled may not be representative of the population. Usually only a small number of gene products are sampled so there is a high variance when the estimated distributions of each are combined. The number of individuals needed to be able to detect reliably a difference between samples is usually unfeasible (Table 5.1).

Secondly, the sample size, in terms of number of loci analysed and number of individuals sampled, affects the variability of estimated allele frequencies (Leberg, 1992) and associated measures of genetic distance (Archie *et al.*, 1989). This has an effect on the stability of phylogenetic relationships derived from replicated samples, and thus on the reliability of the estimate of the phylogeny. The variance in Nei's genetic distance is influenced more by decreasing the number of loci than by decreasing the number of

Power	*dp*	Actual Allele Frequency				
		0.55	**0.70**	**0.80**	**0.90**	**0.95**
50%	0.05	760	645	492	276	146
	0.10	190	162	123	69	50*
	0.20	48	40	31	25	50*
	0.50	6*	9	13	25	50*
80%	0.05	1554	1319	1006	564	299
	0.10	389	332	252	141	76
	0.20	99	82	64	27	50*
	0.50	16	14	13	25*	50*
90%	0.05	2081	1766	1345	756	400
	0.10	520	444	337	189	102
	0.20	132	110	85	50	50*
	0.50	22	20	14	25*	50*

* Sample sizes set to have the minimum expected frequency of 5 per cell required for a χ^2 test for homogeneity.

Above table taken from Baverstock and Moritz (1990), using allele frequencies from Richardson *et al.* (1986).

Table 5.1 The number of individuals needed in each of two samples in order to be able to detect allele frequency differences. The probability of incorrectly rejecting the null hypothesis that the samples are the same is set at 5%. The power of the test is the probability of correctly rejecting the null hypothesis. *dp* is the difference in allele frequency between the two samples. The number of individuals required to detect a given difference depends on the actual frequency of the most common allele.

individuals (Nei, 1978) and therefore it has been recommended that 50 or more loci be used. Unfortunately it can be difficult to resolve large numbers of enzyme systems and the cost and labour required increases with each: most studies, including the present one, make use of only 10-30 loci. If levels of genetic variation are very low, it is difficult to estimate population differentiation and gene flow (Mulvey *et al.*, 1988; Jarne and Delay, 1991; Dybdahl, 1994). The success of allozyme studies depends upon finding at least some genetic variation, which is more difficult with parthenogenetic organisms, and surveying adequate numbers of individuals and loci (*e.g.* Wool *et al.*, 1995; Plague and MacArthur, 1998; Bohonak, 1999b).

Coyne (1982) stressed that the bands (electromorphs) are phenotypes, under which may lie a large amount of cryptic genetic variation, which should be taken into account when interpreting results. Generalisations about heterozygosity and genetic differences among species should be made with caution, emphasising that they are only relative measures. Molecular methods are most effective when they are sensitive enough to pick up a reasonable proportion of the actual variation, and when the genetic variation studied is representative of the genetic character of each population. It is difficult to ascertain whether this is the case (Bossart and Prowell, 1998). The differences between allozymes are not quantified in terms of the number of mutational steps resulting in a given difference. Different proteins may show the same band mobility, and a protein may be generated from different nucleotide sequences. The method also assumes Mendelian inheritance of the observed variable characters, which ought to be tested with breeding studies (*e.g.* Fairbairn and Roff, 1980; Dillon and Wethington, 1994; Roderick, 1996).

5.1.6 Statistical analysis of allozyme data

The statistical analysis of allozyme data will not be described in detail but the most commonly used measures are discussed, with comments on their advantages, drawbacks and assumptions, thus providing the necessary background information for interpretation of the results presented in subsequent chapters. The procedure for analysis of allozyme data begins with the calculation of allozyme (allele) frequencies for each locus in each population. The variability in the allele frequencies is summarised with statistics such as the mean number of alleles per locus (*MNA*), percentage of polymorphic loci at 95% or 99% criterion levels (*P*) and the proportion of individuals heterozygous at each polymorphic locus (*H*). The heterozygosity reported may be the direct count, the expected heterozygosity (from the given allele frequencies in Hardy-Weinberg equilibrium) or Nei's unbiased estimate, which is the expected heterozygosity corrected for sample size (Nei, 1972). Sampling variances (Nei and Roychoudhury, 1974), coefficients of heterozygote deficiency (fixation index) and excess can also be calculated, and linkage disequilibrium is often tested.

Population differentiation and inbreeding of species with patchily distributed populations are described with *F* statistics (Wright, 1943, 1951, 1969) or, occasionally, with *G* statistics (Nei, 1973). These statistics partition variation in the heterozygote deficiency of polymorphic loci into within and between population, and individual, components (Nei, 1977). F_{IS} is the correlation between homologous alleles within individual genotypes, relative to the gene pool of the local population. F_{IT} is the corresponding allelic correlation with reference to the total data set. F_{ST} represents the proportion of the correlation accounted for by the division of the total data set into local populations. Positive values of these fixation indices arise when there are correlations between the genotypes of uniting gametes, that is, heterozygote deficits. A deficiency of heterozygotes is likely to be

due to inbreeding (F_{IS}) or inbreeding combined with population subdivision (F_{IT}). However, when genetic variation is very low, *F* statistics are uninformative (Jarne and Delay, 1991). *F* statistics can also be analysed hierarchically, to partition variation between populations into within and between stream, and catchment, components, for example. Variances of *F* statistics can be calculated by a variety of procedures: the simplest is jack-knifing (Nei *et al.*, 1977; Weir and Cockerham, 1984).

F statistics are based on the island model, where immigrants to any population (deme) are equally likely to come from any other deme, from an infinite or finite number of demes (Wright, 1943). Gene flow is distance-independent. *F* statistics have been modified to accommodate other simple models of population structure. In the stepping stone model, only demes that are immediate neighbours (in one, two or three dimensions) can exchange migrants (Kimura and Weiss, 1964). In the hierarchical model, the demes are arranged into neighbourhoods, and probability of migration is biased towards other demes in the same neighbourhood (Slatkin, 1985b). The above models all commonly assume discrete non-overlapping generations. Continuously distributed populations can also be modelled; these have a more explicit geographical structure (Slatkin, 1985a). The most usual is isolation-by-distance, where populations are uniformly distributed throughout a continuum, and migration is defined by a probability distribution; a migration matrix can also be used (Latter, 1973).

Matrices of pair-wise genetic distance or similarity can be constructed, to provide a single quantitative measure of difference between sets of allele frequencies (Slatkin, 1985a). The most commonly used measure is Nei's genetic distance, which estimates the probability of identity of randomly chosen alleles (Nei, 1972, 1978) but there are several others, for

example Cavalli-Sforza and Edwards (1967) and Rogers (1972). The latter calculate probabilities of differences in heterozygosity, thus they are equivalent to F_{ST}. The distance measures are based on averages across both monomorphic and polymorphic loci, so these should ideally be in the same proportions in each data set when interspecific comparisons are made (Hillis, 1984; Caccone and Sbordoni, 1987). The distance measures also differ with respect to whether they satisfy the triangular inequality (Sneath and Sokal, 1973), which affects their utility in cladogram-building routines (Swofford and Selander, 1981). Sampling variances of genetic distance measures can be calculated and confidence limits constructed (Nei and Roychoudhury, 1974; Mueller and Ayala, 1982). Hillis (1984) sets out the assumptions made when calculating genetic distances.

Gene flow (*Nm*) is the product of the average effective population size and the average number of migrants per population per generation. Gene flow is most usually estimated from its inverse relationship with F_{ST} (Wright, 1969; Felsenstein, 1976); Whitlock and McCauley (1999) discuss problems with this method, arising from the breaking of model assumptions in natural populations. Secondly, it can be estimated using a maximum likelihood method, suitable for large data sets (Slatkin and Barton, 1989). Thirdly, gene flow may be estimated from allele frequency data by calculating the 'conditional average allele frequency' ($p(1)$) of alleles which occur in only one population, and applying the formula of Slatkin (1985b). Computer simulations indicate a strong dependence of $p(1)$ on the overall level of gene flow: $\ln(p(1))$ regresses linearly on $\ln(Nm)$. This method assumes that populations are in gene flow-drift equilibrium, with low mutation rates, and with mutations approximating to an infinite alleles model, and requires a large number of population-specific alleles, preferably at least twenty. Note that current and historical patterns of gene flow cannot be distinguished by either method (Larson *et al.*, 1984; Slatkin,

1987; but see Slatkin and Madison, 1990); this is especially relevant in poorly dispersing species (Caccone and Sbordoni, 1987; Liebherr, 1988). The number of dispersing individuals is often higher than *Nm* implies, as not all will contribute to the gene pool of the population they reach but could be lower, if *Nm* is the result of recent range expansion, for example (Slatkin, 1985a; Bohonak *et al.*, 1998; Bossart and Prowell, 1998).

Population structure can be related to the geographical distribution of the populations, by regression analysis on distance matrices, using Mantel tests (Mantel, 1967; Smouse *et al.*, 1986) or spatial autocorrelation analysis (SAA) (Sokal and Oden, 1978a, b; Stone and Sunnucks, 1993; Arnaud *et al.*, 1999). A Mantel test involves construction of a null distribution by Monte Carlo randomisation; one of the matrices is held constant whilst permutations of the rows and columns of the other are made. The observed test statistic is then compared against this null distribution. In SAA, a spatial correlogram is produced for each allele; this is a plot of correlation co-efficients between sets of localities against a distance measure (Barbujani, 1985; Slatkin, 1985a). No assumptions are made about the process of genetic change or about population history, but these are inferred qualitatively from the slope of the correlogram (*e.g.* a positive correlation may indicate migration) and the similarity or difference between correlograms of different loci (*e.g.* different correlograms may indicate selection at loci, and similar ones, migration). However, in order to infer gene flow, populations must be in gene flow-genetic drift equilibrium.

Significance of most of the above statistics can be tested with non-parametric tests such as χ^2 tests (Lessios, 1992), or hypotheses tested by comparing the observed statistics with a null distribution generated by randomising the data (Emigh, 1980; Smouse *et al.*, 1986).

5.2 Applications of allozyme electrophoresis

5.2.1 Reviews of allozyme studies

Several general reviews of the applications of allozyme studies have been made, focussing on either methodology (*e.g.* Avise 1994; Hoelzel, 1998; Bohonak, 1999a) or synthesising results (*e.g.* Nevo *et al.*, 1984; Jarne and Delay, 1991; Jarne and Städler, 1995). The following sections summarise investigations of variation on a number of scales: from phylogeny, through speciation, geographic patterns in intraspecific variation and population structure, to dispersal and mating systems. This review illustrates the current state of knowledge about processes affecting freshwater invertebrates gained from allozymes, but also highlights the interpretative difficulties that arise due to the mismatch between the theoretical models on which analyses are based and the particular circumstances of the species under investigation.

5.2.2 Phylogeny

Allozymes may provide a more quantitative and objective method of reconstructing phylogeny than morphological characters, which may have been subject to stronger selection. Matrices of similarity or distance coefficients can be presented as dendrograms and then compared with other classifications (Avise, 1975). It is important to note the difference between character state phylogeny and taxon phylogeny, and that not all molecular characters are equally informative about the taxon phylogeny, for example some may be the product of convergent evolution (Avise, 1983).

Classifications based on allozyme data have been compared with traditional morphological classification (Chambers, 1980; Bulnheim and Scholl, 1981; Caccone and

Sbordoni, 1987). Enzyme variation can also be used to provide confirmation of taxonomic relationships based on morphological characters (Zurwerra *et al.*, 1986). Allozyme analysis shows the relationship between species better than studies of natural hybridisation when the species group shows a high degree of single-island or island-group endemism and limited sympatry, because geographical isolation in the wild does not necessarily involve reproductive isolation (Pashley *et al.*, 1985). Different amounts of genetic variability within related species may also be used to infer their phylogeny (Malacrida *et al.*, 1996). Genetic variation in the particular case of 'phylogenetic relict' species has been employed to reveal whether the conserved morphology is the result of life in a stable niche, genetic homeostasis or the loss of mutability (Selander *et al.*, 1970).

5.2.3 Speciation

The frequency distributions of allozymes can be used to distinguish morphologically similar species, especially when they occur sympatrically (Ayala, 1983). Fixed allelic differences (*e.g.* Hefti *et al.*, 1988; Nilsson *et al.*, 1988; Sweeney and Funk, 1991; Milankov *et al.*, 2000) or a combination of fixed allelic differences and relative genetic distances (Dillon and Davis, 1980; Funk *et al.*, 1988; Jackson and Resh, 1992) can be used as evidence. Comparison of interspecific and intraspecific genetic distance measures alone may also distinguish species in cases where species pairs have been isolated longer than population pairs (Caccone and Sbordoni, 1987; Sweeney *et al.*, 1987; Clarke *et al.*, 1998). Where there is no evidence of gene flow even between adjacent populations of the different forms, this can be used to elevate previously recognised races or subspecies to species level (Byrne and Nichols, 1999).

The process of speciation has also been investigated using allozymes. Nei's (1972) genetic distance correlates approximately with time since divergence of a lineage so a rough date can be put to speciation events (Pashley *et al.*, 1985; Lees and Ward, 1987). Speciation through geographic isolation is particularly suited to allozyme investigations (Ponder *et al.*, 1994). Ponder *et al.* (1994) found evidence for both allopatric and sympatric speciation in their study of Hydrobiidae (Gastropoda) in southeast Australia. However, when speciation is rapid, little genetic differentiation is usually observed between closely related species (Ayala, 1983; Clarke *et al.*, 1998). For example, the genus *Partula* (Gastropoda: Partulae) has speciated on the island of Moorea (South Pacific) after relatively simple genetic changes (Johnson *et al.*, 1984).

Hybrids can be detected from allozyme variation, including the hybrid origin of species, inferred when loci are fixed in heterozygous condition, being heterozygous for alleles diagnostic of the supposed parental taxa (Bullini, 1983; Wolf, 1987). Conversely, loci that are differently fixed are evidence against hybridisation (Funk and Sweeney, 1990). Reproductive compatibility can be studied directly and compared with genetic differentiation (Johnson *et al.*, 1984; Clarke *et al.*, 1998). The maintenance of reproductive compatibility in some species despite large-scale genetic divergence, low dispersal ability and local selection pressures illustrates the difficulty of viewing speciation as a purely genetic process (Johnson *et al.*, 1984).

5.2.4 Geographical variation and population structure

Allozyme studies can reveal the spatial and temporal genetic structure of populations. Structure arises from patterns of mating within populations, expressed as a deviation from Hardy-Weinberg equilibrium, and from the amount of gene exchange

between populations, expressed as a variance of allele frequencies among populations (Saura, 1983). The deviations and variances are then tested against a hypothesis of neutrality (Kimura, 1991). In invertebrate populations, the proportion of monomorphic loci is 25-75%, but with these loci tending to be the same in different populations of the species, so the population structure is usually detected in the allele frequencies of the polymorphic loci (Avise, 1994).

Genetic variation amongst populations of a species is dictated by a combination of spatially varying selection pressures, neutral evolution and gene flow. Spatially varying selection pressures produce different allele frequencies in different regions or habitats. Neutral variation between populations arises from the random sampling of gametic genotypes in finite populations (*i.e.* genetic drift), population bottlenecks, founder events and the background neutral mutation rate (Fuerst *et al.*, 1977; McCommas and Bryant, 1990; Kimura, 1991). Population differentiation is opposed by gene flow: this is dependent upon species' dispersal ability relative to the physical isolation of populations and population history (Caccone and Sbordoni, 1987; Slatkin, 1987; Liebherr, 1988).

Models of population structure approximate these complexities by envisaging populations as isolated and island-like. Kimura and Weiss (1964) expanded the stepping-stone model to demonstrate that, assuming populations have a uniform distribution in space and time, genetic differentiation among local populations is indicative of extensive macrogeographic variation. A lack of genetic differentiation locally is associated with macrogeographic genetic uniformity. This is not necessarily the case when populations are transient at the local scale but the species persists in the area at a larger scale. In this case

locally differentiated populations can arise through founder events whilst the area and regional gene pools remain more uniform over space and time (Hebert, 1974).

The studies described below use allozymes to investigate geographical population structure on a range of scales from tens of metres to thousands of kilometres, as applied to selected Canarian species in the present study. They variously found that the observed patterns of population differentiation and geographic variation were due to: (1) stochastic processes such as the history of population extinction, colonisation and bottlenecks; (2) deterministic factors such as dispersal ability, environmental barriers to dispersal, habitat patchiness, colonisation routes; and (3) selection pressures.

Many studies conclude that stochastic processes are sufficient to explain the geographical variation and population structure in the study organisms without ruling out spatial variation in selection pressures (Table 5.2). In these studies, populations did not generally sort according to geographical proximity and little evidence of macrogeographic clines of allele frequencies among conspecific populations was found. Allele frequencies were influenced by random factors such as fluctuations in population size and founder events.

Several factors may lead to a pattern of population differentiation that is not correlated with geographic isolation. Variation in effective population size and environmentally induced bottlenecks may give a false impression of genetic distance between sites due to associated change in allele frequencies (Jackson and Resh, 1992; Colgan and Ponder, 1994; Schug *et al.*, 1998). Low genetic distances between populations may reflect recent isolation rather than enhanced dispersal (Hughes *et al.*, 1996).

Reference	Species	Group/Order and Family
Hebert, 1974	*Daphnia magna*	Cladocera, Daphniidae
Varvio-Aho, 1979	*Gerris* spp.	Hemiptera, Gerridae
Varvio-Aho and Pamilo, 1979	*Gerris lacustris*	Hemiptera, Gerridae
Varvio-Aho and Pamilo, 1980	*Gerris lateralis* *G. odontogaster*	Hemiptera, Gerridae
Varvio-Aho and Pamilo, 1981	*Gerris lateralis* *G. odontogaster*	Hemiptera, Gerridae
Varvio-Aho, 1981	*Gerris* spp.	Hemiptera, Gerridae
Hebert and Payne, 1985	*Mesostoma lingua*	Turbellaria, Rhabdocoela
Agatsuma, 1987	*Simulium ochraceum*	Diptera, Simuliidae
Sweeney *et al.*, 1987	*Ephemerella* spp. *Eurylophella* spp.	Ephemeroptera, Ephemerellidae
Funk *et al.*, 1988	*Eurylophella* spp.	Ephemeroptera, Ephemerellidae
Boileau *et al.*, 1992	various species	Anostraca Cladocera Collembola Copepoda Notostraca Ostracoda Turbellaria
Jackson and Resh, 1992	*Helicopsyche borealis*	Trichoptera, Helicopsychidae
Bunn and Hughes, 1997	*Baetis* sp.	Ephemeroptera, Baetidae
	Paratya australiensis	Decapoda, Atyidae
	Rheumatometra sp.	Hemiptera, Gerridae
	Tasiagma ciliata	Trichoptera, Tasimiidae
Hughes *et al.*, 1998	*Tasiagma ciliata*	Trichoptera, Tasimiidae
Byrne and Nichols, 1999	*Culex pipiens*	Diptera, Culicidae

Table 5.2 Allozyme studies on freshwater invertebrates where population differentiation was attributed to stochastic processes.

Populations may not have reached gene flow/drift equilibrium at the scale of the study, particularly if they are ephemeral (Arnaud *et al.*, 1999; Franceschinelli and Kesseli, 1999). Finally, long distance dispersal might be as probable as short distance dispersal, especially where any kind of dispersal is a rare event (Bohonak, 1999b). This is envisaged to be the case for the passively dispersed freshwater mollusc *Ancylus striatus* (Mollusca: Ancylidae) on the Canary Islands (Chapter 8).

Genetic drift is a sufficient explanation for genetic variation where effective population size relates to genetic differentiation (Varvio-Aho and Pamilo, 1980; Varvio-Aho, 1981). When measurements of N_E vary across populations and temporally, drift and founder effects must be important factors in producing the genetic structure (Agatsuma, 1987; King, 1987; le Gorre and Kremer, 1998). Population bottlenecks and founder events are a likely explanation for the linkage disequilibrium that is often observed (Smith and Fraser, 1976; Hebert and Moran, 1980; Siegismund and Muller, 1991). N_E can also explain population genetic structure in combination with factors such as isolation and dispersal ability (Varvio-Aho, 1983).

The number and geographic spread of the set of potentially inter-breeding individuals is increased if a population is part of a metapopulation system (Seppä and Laurila, 1999). In a metapopulation, founder effects may enhance or decrease genetic variation among groups (McCauley, 1991; Harrison and Hastings, 1996). The resulting structure is dependent on factors such as the number and origin of founding individuals, the population extinction rate and the number of habitat patches (Wade and McCauley, 1988; le Gorre and Kremer, 1998). Recent genetic models predict increased differentiation under most circumstances because recolonisation events produce the founder effects of reduced

genetic diversity within, and increased differentiation between, patches. It is predicted that younger populations are more differentiated than older populations. However, reduced variation within the metapopulation may result if the recolonisation process results in homogenising gene flow (Hebert, 1974; Dybdahl, 1994).

Stochastic founder events can be very influential in producing genetic differentiation and can reinforce population isolation (*e.g.* Hebert and Moran, 1980; Schug *et al.*, 1998; Byrne and Nichols, 1999). For example, low heterozygosity in *Gerris odontogaster* (Hemiptera, Gerridae) was explained by repeated founder events resulting from the temporary nature of its pond habitat (Varvio-Aho, 1979). Given large population sizes, and particularly in non-outcrossing organisms, it may take thousands of generations for populations to overcome the effects of founder events and reach genetic equilibrium (Boileau and Hebert, 1988; Boileau *et al.*, 1992). The time elapsed since populations shared a common ancestor is also important (Dillon, 1984, 1988).

Many studies on freshwater invertebrates have found that population differentiation is spatially structured (Table 5.3). In these studies, deterministic processes (resulting from dispersal ability, habitat structure and spatial variation in selection pressures) are invoked, in addition to stochastic processes such as genetic drift and founder effects.

Geographical isolation of populations may have an important influence on population genetic structure. Isolation may correlate with population and gene differentiation and standardised variances of allele frequencies both within and between species (*e.g.* Caccone, 1985; Caccone and Sbordoni, 1987; Goudet *et al.*, 1994). It is expected that for Canary Island trichopteran species (actively dispersing), population

Reference	Species	Group/Order and Family
Varvio-Aho *et al.*, 1978	*Gerris* spp.	Hemiptera, Gerridae
Gooch and Hetrick, 1979	*Gammarus minus*	Amphipoda, Gammaridae
Chambers, 1980	*Goniobasis* spp.	Gastropoda, Hydrobiidae
Gooch and Golladay, 1981	*Gammarus minus*	Amphipoda, Gammaridae
Zera, 1981	*Aquarius remigis* *Limnoporus canaliculatus*	Hemiptera, Gerridae
Varvio-Aho, 1983	*Gerris* spp.	Hemiptera, Gerridae
Gooch and Glazier, 1986	*Gammarus minus*	Amphipoda, Gammaridae
Brown and Richardson, 1988	*Lymnaea elodes*	Gastropoda, Lymnaeidae
Mulvey *et al.*, 1988	*Biomphalaria* spp.	Gastropoda, Hydrobiidae
Sperling and Spence, 1990	*Limnoporus* spp.	Heteroptera, Gerridae
Siegismund and Muller, 1991	*Gammarus fossarum*	Amphipoda, Gammaridae
Preziosi and Fairbairn, 1992	*Aquarius remigis*	Hemiptera, Gerridae
Colgan and Ponder, 1994	*Fluvidona* spp. *Fonscochlea* spp. *Trochidrobia* spp.	Gastropoda, Hydrobiidae
Ponder *et al.*, 1994	*Fluvidona* spp.	Gastropoda, Hydrobiidae
Dillon and Wethington, 1995	*Physa heterostropha*	Gastropda, Physidae
Hughes *et al.*, 1996	*Caridina zebra*	Decapoda, Atyidae
Bohonak, 1999b	*Arrenurus* spp.	Acari, Hydrachnida
Hughes *et al.*, 1999	*Yoraperla brevis*	Plecoptera, Peltoperlidae

Table 5.3 Allozyme studies on freshwater invertebrates where population differentiation was due to deterministic as well as stochastic processes.

differentiation will increase with geographical isolation (Chapters 6 and 7). Populations isolated on the edge of a species' range have reduced genetic variation, according to theoretical models of isolation-by-distance (Wright, 1943) and the one-dimensional stepping-stone model (Kimura and Weiss, 1964). This is not always the case, however, as genetic variation could be increased by the greater environmental heterogeneity of marginal habitats (Gooch and Glazier, 1986, Guinand, 1994). Van Dongen *et al.* (1998) found that populations in isolated habitat fragments had less genetic diversity than those in continuous areas of habitat or in less isolated fragments. Pairs of disjunct and contiguous populations of *Collops georgianus* (Coleoptera: Melyridae) showed an effect of isolation by distance but not of habitat continuity on population differentiation (King, 1987), whilst Johnson and Black (1991, 1995) found that discontinuities in habitat were important barriers to gene flow for molluscs. The patchy distribution of streams on the Canary Islands, and the nature of oceanic islands, is anticipated to have a profound effect on the genetic structure of the species present: population differentiation is expected to be enhanced and genetic variation within populations reduced relative to continental species/populations inhabiting a more continuous environment (Chapter 6).

Spatial variation in population genetic structure also includes the phenomenon of 'area effects', where allele frequencies remain constant over an area larger than the panmictic unit does, and areas of similar allele frequencies are separated by steep clines from neighbouring areas. Area effects may arise from local bottlenecks, but do not seem to be cases of incipient speciation: they were first described for *Cepaea* (Gastropoda: Helicidae), a genus in which only four allopatric species are recognised (Selander and Ochman, 1983). The pattern is also predicted by a spatial model with multiple stable solutions (Keeling,

1999), for example, when all homozygotes are superior to all heterozygotes. The spatial element in the model prevents any particular homozygote genotype becoming fixed.

It is expected from the theoretical models that populations in closer proximity to one another will be less differentiated than those further apart, and that population differentiation will be increased at higher hierarchical levels (Wright, 1943, 1951; Kimura and Weiss, 1964). The hierarchical nature of spatial genetic variation can be addressed with an appropriate sampling scheme, for example, one designed for comparison of sites within and between streams, and of streams within and between catchments (*e.g.* Hughes *et al.*, 1995, 1996; Bunn and Hughes, 1997). The hierarchy can include sites within and between real or habitat islands (*e.g.* Mulvey *et al.*, 1988; Dillon and Wethington, 1995; Johanneson and Tatarenkov, 1997).

The relationship between genetic structure and patterns of habitat geographic structure other than linear geographical proximity can be studied (Caccone, 1985; Caccone and Sbordoni, 1987; Hughes *et al.*, 1999). For example, the genetic structure of *Gammarus fossarum* (Amphipoda: Gammaridae) showed a tree-like pattern of similarity between populations that corresponded with the branching pattern of the river system in which they were sampled (Siegismund and Muller, 1991). Potential barriers to dispersal can also produce strongly environmentally correlated patterns of genetic differentiation (Gooch and Hetrick, 1979; Siegismund and Muller, 1991). Within streams, larger waterfalls and cascades can be a barrier to movement (Hughes *et al.*, 1996), whilst active dispersal between streams is not possible for species aquatic at all stages of their life history (Gooch and Golladay, 1981).

Significant correlations between genetic and geographic distances have been found in a minority of cases (*e.g.* Knoll and Rowell-Rahier, 1998; Raspé and Jacquemart, 1998; Raybould *et al.*, 1998), and are expected for actively dispersing Trichoptera (Chapters 6 and 7). Connectivity networks (a tool for the analysis of spatial autocorrelation) may correlate better with genetic differentiation than geographic distances or a hierarchical classification of sampling sites (Arnaud *et al.*, 1999). Large differences in allele frequencies at even just one or two loci are strong evidence for low gene flow, but homogeneity of populations is weaker evidence for high gene flow, especially when few populations are sampled (Johnson and Black, 1995, 1996). Dispersal ability underpins the scale at which populations are differentiated. In poorly dispersing organisms, a high degree of genetic divergence is expected between populations in close proximity due to low gene flow (*e.g.* Brown and Richardson, 1988; Colgan and Ponder, 1994; Ponder *et al*, 1994).

Variations in habitat continuity over time may also affect population genetic structure. Hebert (1974), for example, found no significant differences in allele frequencies between populations of *Daphnia magna* in intermittent and permanent ponds within the same localities. This was probably because colonisation events homogenised the populations. A study on the effect of habitat permanency on the population differentiation of *Gerris* species was inconclusive as it was confounded with characteristics such as wing length, which varied with habitat (Varvio-Aho, 1979, 1983). Habitat stability in terms of disturbance frequency can also influence genetic structure. For example, allozyme variation in *Baetis tricaudatus* (Ephemeroptera: Baetidae) and *Hesperoperla pacifica* (Plecoptera: Perlidae) from a stream with constant flow and a stream with seasonally varying flow rate demonstrated that genetic variability in both species was higher in the more variable environment (Robinson *et al.*, 1992).

Temporal variation in population genetic structure has been studied by comparing monthly (*e.g.* Smith and Fraser, 1976; Young, 1979; Hebert and Moran, 1980) or yearly (*e.g.* Sweeney and Funk, 1991; Hughes *et al.*, 1995; van Dongen *et al.*, 1998) samples. Preziosi and Fairbairn (1992) found significant temporal variation in allele frequencies in *Aquarius remigis* (Hemiptera: Gerridae) that they attributed to population bottlenecks through over-wintering mortality. Four possible explanations for temporal variation in *Daphnia magna* (Cladocera: Daphniidae) were suggested by Berger and Sutherland (1978): temporal fluctuations in selection pressures (Hedrick *et al.*, 1976); changes in fecundity of parthenogenetic genotypes; recruitment from dormant propagules; and the sampling of a 'mosaic' of clonal genotypes in differing proportions on different occasions. Müller and Seitz (1994) attributed temporal variation in *Daphnia* population allele frequencies to differential seasonal mortality of different genotypes, for example through variation in temperature tolerance (Carvalho and Crisp, 1987).

Allozyme studies have proved useful in reconstructing large-scale distribution changes, for example during post-Pleistocene recolonisation. Colonisation by successive founder events is predicted to lead to a pattern of genetic diversity decreasing with distance from the source region (Gooch and Glazier, 1986; Gasperi *et al.*, 1991; Stone and Sunnucks, 1993), with different alleles becoming fixed in different colonisation episodes (García-Marín *et al.*, 1999). Loss of variation at the edge of the species range has also been found in other studies (Coutellec-Vreto *et al.*, 1994, Vrijenhoek and Graven, 1992). Post-glacial colonisation routes and range expansions from refugia may be distinguishable even in cases where there is high gene flow (Stauffer *et al.*, 1999). On a lesser timescale, the spread of introduced species can be traced (Woodruff *et al.*, 1985).

Some studies have demonstrated that spatial variation in selection pressures is necessary to explain geographical population structure (Jokela *et al.*, 1999); however, selection is not the most parsimonious explanation when the variation can also be explained by equilibrium between genetic drift and gene flow. Significant linkage disequilibria are sometimes found, in which case and selection may be acting on the allozymes themselves or on a gene with which they are linked (Siegismund and Muller, 1991). Ongoing selection is an unlikely explanation in populations that undergo periodic bottlenecks, as small populations would be unable to support a 'selectional load', resulting in local extinction.

The choice of geographic scale for a study affects its interpretation. Sampling at small spatial scales can have the consequence that the specimens collected are members of one or a few sibling/half-sib groups, which can lead to problems in the analysis and interpretation of results. This gives the, apparently paradoxical, result that genetic differentiation is greater at lower than at higher hierarchical levels. This occurs when the scale of sampling does not coincide with the scale of the panmictic population and when offspring from different matings are not randomly distributed (Varvio-Aho and Pamilo, 1981; Giles *et al.*, 1998; Knoll and Rowell-Rahier, 1998). This phenomenon additionally indicates that the majority of the siblings do not disperse more than a few metres, and that the females determine the distribution of genotypes by their choice of oviposition site (Schmidt *et al.*, 1995; Bunn and Hughes, 1997), or location in the case of plants. There is also reduced likelihood of finding alleles in Hardy-Weinberg equilibrium (Schmidt *et al.*, 1995).

Inadvertent pooling of several populations into single samples also complicates data analysis (Colgan and Ponder, 1994; Plague and McArthur, 1998). Subdivided populations

have reduced heterozygosity, through the Wahlund effect (Lees and Ward, 1987; Arnaud *et al.*, 1999). The Wahlund principle (also called isolate breaking) is that the average homozygosity decreases when subpopulations join (Hartl and Clark, 1997). When populations have no further significant subdivision, this depression of heterozygosity does not occur, and loci tend to conform to Hardy-Weinberg equilibrium expectations (Hughes *et al.*, 1996).

When studies are conducted over large geographical scales, or over a range of scales up to hundreds of kilometres or more (Chambers, 1980; Mulvey *et al.*, 1988) the use of unmodified island models (Wright, 1951, 1969) to estimate gene flow is inappropriate. This is because dispersal among populations is very unlikely to be equal or symmetric (Boileau *et al.*, 1992).

5.2.5 Dispersal

Dispersal patterns affect the genetic structure of populations by determining gene flow, hence genetic structure can be used to infer dispersal patterns (Avise, 1992). Allozyme studies of dispersal bridge the gap between ecology and evolution. Understanding of microevolution requires investigation of how the movement of genes among populations interacts with genetic drift, natural selection and mutation (Bohonak, 1999a). Bohonak (1999a) quantified the relationship between dispersal ability and spatial scale over which populations differ genetically for a range of vertebrates and invertebrates. Population differentiation can reflect a number of processes, including changing effective population size, natural selection on the markers surveyed, the history of the distribution range of the species (vicariance) and the history of gene flow between the populations (Slatkin, 1985a). Comparative studies should aim to eliminate the effects of these alternative processes.

Wing-polymorphic Gerridae provide an ideal opportunity to study the effect of different dispersal ability on population structure (Varvio-Aho *et al.*, 1978; Varvio-Aho and Pamilo, 1979). It is expected that more brachypterous species will show greater population differentiation than those with greater flight ability, due to a lack of interpopulation dispersal (Zera, 1981; Varvio-Aho, 1983). However other factors which differ among the species, such as permanency of habitat (Varvio-Aho, 1979) or population isolation (Sperling and Spence, 1990), may explain the population structure or mask any effects of wing-length polymorphism. Allozyme frequencies can also be related to mark-recapture studies of dispersal (Varvio-Aho and Pamilo, 1981). Waples (1987) found a significant negative correlation between population genetic structure (D and F_{ST}) and ranked dispersal ability in ten species of marine shore fishes from southern California. The species are, however, not ecologically or phylogenetically comparable, as they are associated with a range of habitats and represent nine different families. Measures of population structure are sensitive to the effects of natural selection and historical factors on allozyme frequencies but Waples reasoned that there was no *a priori* reason to invoke these additional factors. Within the five orders of meiofauna studied by Boileau *et al.* (1992), no correlation was found between F_{ST} and an index reflecting dispersal ability. Instead, founder effects were emphasised, particularly where one or a few parthenogenetic individuals may have founded populations.

Bunn and Hughes (1997) applied the comparative approach to aquatic and semi-aquatic invertebrates of Australian streams. Greatest genetic differentiation was found between populations of aquatic taxa at a fine scale (that is, within reaches, streams and sub-catchments), indicating limited in-stream movement by larval stages and aquatic species such as *Caridina zebra* (Decapoda: Atyidae). Amongst the semi-aquatic insects, the

principle mechanism of dispersal is active flight by adults and local genetic variation was the result of the combined dispersal ability of the larval and adult stages and the stochastic effect of the distribution of oviposition sites chosen by females (Bunn and Hughes, 1997; Hughes *et al.*, 1998).

Parasitic and non-parasitic species may differ in dispersal potential, and thus make another good subject for comparative studies. Sister species of *Arrenurus* (Acari: Hydrachnida) differ only in that the parasitic species use their dipteran and odonate hosts as vectors for dispersal. Bohonak (1999b) hypothesised that the loss of a parasitic life history strategy increased population differentiation by reducing dispersal. Historical biogeographic and selective explanations for population differentiation are eliminated from this comparison by the choice of closely related, regionally sympatric species, which are therefore assumed to have a shared history. Loss of parasitism was, in fact, only weakly associated with increased population differentiation and reduced heterozygosity in *Arrenurus*, but in the majority of species studied both heterozygosity and population differentiation were low, obscuring the effects of dispersal ability. Comparison with *Unionicola* (Hydrachnida) which occupies a more southerly region (Edwards and Dimmock, 1997) suggests that *Arrenurus* has not reached gene flow/drift equilibrium due to the relatively short time elapsed since glacial retreat in the northern states. Therefore, despite the careful choice of study organism no firm conclusion could be reached about the effect of dispersal on population differentiation of parasitic and free-living mites.

Allozyme studies can also be designed to make inferences about potential dispersal mechanisms. The matrix of genetic distances between populations of *Physa heterostropha* (Pulmonata: Physidae) on estuarine islands correlated significantly with the matrix of

geographical distances, irrespective of water barriers (Dillon and Wethington, 1995). This suggested that passive dispersal by seabirds is more effective than active dispersal by crawling overland. In aquatic insects, the relative importance of larval dispersal (by crawling and drift) and adult dispersal (by flight) was tested with *Baetis* sp. (Ephemeroptera: Baetidae) (Schmidt *et al.*, 1995). If flight is the primary mechanism then isolation-by-distance both within and across drainages is predicted (as for Canarian Trichoptera, Chapters 6 and 7), whilst if dispersal occurs principally in the larval stage then distant populations in the same drainage are expected to be more similar than neighbouring populations in different drainages. A general lack of population differentiation at any hierarchical level was found, which suggested that flight is an important dispersal mechanism, but the predictions were not borne out exactly. The samples seemed to consist of sibling groups (indicative of low levels of larval dispersal) so greater differentiation than expected was found at small spatial scales.

The relationship between genetic differentiation and distributional range has also been investigated, testing the hypothesis that species with a greater range size have less population differentiation, which would be the case if they were better dispersers. The findings of Plague and McArthur (1998) for Trichoptera were ambiguous, with the correlation of increasing genetic differentiation with decreasing range size being due to one data point. No correlation between range size and genetic divergence was found for pond meiofauna, suggesting that factors other than dispersal ability determine the species distributions (Boileau and Hebert, 1988; Boileau *et al.*, 1992).

5.2.6 Natural selection acting on allozymes

Selection is potentially an important factor in maintaining allozyme variation (*e.g.* Johnson and Black, 1991; Nevo *et al.*, 1994; Kreitman and Akashi, 1995), especially in the case of heterosis ('hybrid vigour') - there is often a positive correlation between multi-locus heterozygosity and fitness (Zouros and Pogson, 1994). When the influence of natural selection on allozymes is tested for, neutral variation is the null hypothesis to be rejected (Avise, 1994). In order to detect the effect of selection the study usually has to be designed with that particular aim, and should involve validation with laboratory work on allozyme properties and fitness effects (Hedrick *et al.*, 1976; Zera, 1987; Nevo *et al.*, 1994).

The influence of selection on allele frequencies at specific loci can be recognised if allele frequencies at homologous loci in closely related sympatric species are correlated (excluding the possibility of hybridisation) (Varvio-Aho and Pamilo, 1982). No evidence of selection was found by this criterion at all but one of the 45 loci studied in two natural populations of *Drosophila*, though this was refuted by Borowsky (1982). Smith and Fraser (1976) favoured natural selection acting on co-adapted gene combinations (Kreitman and Akashi, 1995) to explain the marked linkage disequilibrium which they found in *Simocephalus serrulatus* (Cladocera: Daphniidae), as this is more likely than direct linkage between loci, and a co-adapted gene complex can be maintained in a parthenogenetic organism. The action of natural selection is also suggested by inconsistency in the pattern of population differentiation from locus to locus (Slatkin, 1987).

Clines in allele frequencies are suggested to be the result of natural selection, with varying selection pressure along an environmental gradient (*e.g.* Nevo *et al.*, 1986; Sweeney *et al.*, 1986; Nevo *et. al.*, 1994). However, geographically structured neutral gene flow

could also produce the same result in some cases (Agatsuma, 1987; Dillon, 1984; Johnson and Black, 1995). Knowledge of the known functional properties of enzymes can be used to interpret genetic variation in relation to environmental gradients (Verspoor, 1983; Qian and Davies, 1996; Johanneson and Black, 1999). Selection pressures can result in convergence of isolated populations that have to cope with the same environmental factors; however, if environmental conditions are particularly variable, adaptation may be facultative (through phenotypic plasticity) rather than constitutive (Qian and Davies, 1996). In contrast, habitat differences may account for a significant proportion of allozyme variation (Hedrick, 1986; Johnson and Tatarenkov, 1997). Selection may also cause differences in gene diversity between species, depending upon niche width, environmental stability and dispersal ability (Dillon and Davis, 1980; Lees and Ward, 1987; Coutellec-Vreto *et al.*, 1994).

Suspected selection can be tested with fitness experiments in the laboratory, though most studies stop short at describing environmental correlations. Temperature-dependent kinetic variation among PGI (phosphoglucose isomerase) allozymes from *Limnoporus canaliculatus* (Hemiptera: Gerridae) was consistent with latitudinal variation in allozyme frequencies (Zera, 1987). Analysis of reproductive components of fitness, estimated for the brackish water species *Sphaeroma rugicauda* (Isopoda: Sphaeromatidae), showed that balancing selection maintains both alleles of the diallelic PGI locus (Heath *et al.*, 1988). The logical progression from these studies is to investigate the physiological effects of allozyme variation and fitness differences between individuals with different genotypes, to show that the kinetic variation is actually subject to selection in natural populations, and to confirm that the selective pressure is acting on the allozymes themselves rather linked loci (Zera, 1987).

Allozyme studies can be used to test for the presence of genetic variation underlying ecophenotypes, *i.e.* whether the phenotypes have different genotypes, due to selection, or not. The role of selection is not always supported. For example, Carvalho (1987) and de Meester (1994) found allozyme differences between *Daphnia magna* (Daphniidae) clones with different physiological tolerances and behavioural strategies. Gooch and Hetrick (1979) found that most populations of a given eco-phenotype in a given area were genetically similar (a 'neighbourhood effect'). However, populations of the same eco-phenotype were not more similar than the population at large, so gene flow and random drift were sufficient explanation for the neighbourhood effect; selection need not be invoked. Sweeney *et al.* (1986) found significant genetic differentiation within one species of Ephemerellidae (Ephemeroptera) that exhibits eco-phenotypes, but not in another. The variation was possibly due to a spatial cline in selection pressures, and so the concept of the eco-phenotype is supported.

5.2.7 Breeding systems

The breeding system of a species, whether self-fertilisation, outcrossing or asexual reproduction, has a profound effect on patterns of variation (Hebert, 1987; Carvalho, 1994). Allozyme studies can therefore be used to deduce breeding system (*e.g.* Brown and Richardson, 1988; Chaplin and Ayre, 1989; Vrijenhoek and Graven, 1992), and this is utilised in the study of *Ancylus striatus* on the Canary Islands (Section 8.4.5). For example, self-fertilising and parthenogenetic species tend to have fewer polymorphic loci and lower heterozygosity than outcrossing species (Suomaleinen *et al.*, 1976; Jarne *et al.*, 1993; Jarne and Städler, 1995). When species are known to be parthenogenetic, parent-offspring analysis can be used to determine whether parthenogenesis is mictic (*i.e.* involving meiosis) or apomictic (*e.g.* Berger and Sutherland, 1978; Schwartz and Hebert, 1987). The

polyploidy that is often associated with parthenogenesis increases the tendency of populations to become genetically uniform, by slowing the spread of mutations and by decreasing the chance of expression of new mutations (Suomaleinen *et al.*, 1976). However, a number of studies have found that parthenogenesis does not lead to genetic homogeneity (*e.g.* Smith and Fraser, 1976; Livshits *et al.*, 1984).

In Mollusca, the selfing rate has been shown to vary between individuals and populations (Jarne and Städler, 1995). The frequency of self-fertilisation can also be estimated by parent-offspring analysis, looking for segregating polymorphisms that positively identify outcrossing (*e.g.* Karlin *et al.*, 1980; Mulvey and Vrijenhoek, 1981; Woodruff *et al.*, 1985; Jarne *et al.*, 2000). An alternative approach to estimating the self-fertilisation rate is to infer it from the inbreeding co-efficient (F_{IS}) assuming mixed mating and genetic equilibrium. The Wahlund effect or biparental inbreeding may also cause positive values of F_{IS}, due to heterozygote deficiency (Chaplin and Ayre, 1989; Jarne and Städler, 1995), but the higher the actual selfing rate the less important are these sources of error. An advantage to this population-based approach is that selfing rates are averaged over several generations. This method is not effective if inherent levels of polymorphism are very low.

Where the breeding system is known, its effects on the population structure may be investigated (Peakall and Beattie, 1991). Genetic variation in parthenogenetic and bisexual populations of a freshwater snail was compared by Livshits *et al.* (1984). The parthenogenetic populations were found to have more fixed population-specific alleles leading to greater population genetic differentiation yet with less genetic variation. Other studies have found more limited population differentiation and genetic stability over time in

parthenogenetic populations (Hebert and Moran, 1980). Genetic divergence due to population isolation often leads to reproductive isolation (Jarne and Städler, 1995) but the degree of reproductive isolation is not always related to genetic divergence (Johnson *et al.*, 1984) or geographical distance (Bauer and Bauer, 1992). Allozymes were used to compare the population structure of an autogenous and an anautogenous species of *Simulium* (Diptera: Simuliidae) (Snyder and Linton, 1984). The latter is expected to disperse more before oviposition, increasing the potential size of the panmictic population. However, the results of allozyme analysis and karyotyping were not in agreement. The population structure often reflects the pattern of the environment in animals with apomictic parthenogenesis, or automixis with a mechanism to introduce heterozygosity (Saura, 1983). Typically, a population structure of a set of genotypes arranged along an environmental gradient, each with slightly different adaptations, is produced (Suomaleinen *et al.*, 1976; Selander *et al.*, 1978; Jokela *et al.*, 1999).

5.2.8 Genetic variation in disease vectors

Finally, particular attention is drawn to work on freshwater species that are vectors for agents of disease, providing an applied focus for studies of genetic variation. These include species of *Biomphalaria* (Gastropoda: Planorbidae), which are vectors of schistosomiasis, Simuliidae, which are vectors of onchocerciasis, and Culicidae, which transmit a number of tropical diseases including malaria. Studies have focussed on vector species identification (Matthews and Munstermann, 1983; Bandoni *et al.*, 1995a, b), correlations between genotype and parasite frequency (Agatsuma, 1987) and population-level host-parasite interactions (Vrijenhoek and Graven, 1992). The latter consider the influence of parasites on population structure (Mukaratirwa *et al.*, 1996a; Jokela *et al.*, 1999) and the evolution of mating systems (Jarne and Delay, 1991). Vector population

structure, dispersal/colonisation ability and breeding system have been studied to assess the risk of disease spreading to new areas (*e.g.* Snyder and Linton, 1984; Woodruff *et al.*, 1985; Kambhampati *et al.*, 1990; Mukaratirwa *et al.*, 1996b; Pointier, 1999).

Chapter 6

Population Structure and Dispersal of

Mesophylax aspersus

(Trichoptera: Limnephilidae)

Population Structure and Dispersal of *Mesophylax aspersus* (Trichoptera: Limnephilidae)

SUMMARY

Population genetic structure of the circum-Mediterranean caddisfly *Mesophylax aspersus* (Trichoptera, Limnephilidae) on the Canary Islands was investigated by studying allozyme variation at nine putative loci in five populations. Genetic variability, population structure and gene flow were compared with data in the literature for continental taxa to assess the effect of isolation of island populations on the species' genetic structure. Larvae were collected from streams on the islands of Tenerife (one population), La Gomera (two populations in the same catchment) and La Palma (two populations in different catchments). Genetic variability within populations was high relative to that recorded previously for continental Trichoptera, *e.g.* mean heterozygosity was 0.119-0.336 (0.035-0.15 in continental taxa). Highly significant population structure was observed (multilocus F_{ST} = 0.250), and there was significant within-population structuring (multilocus F_{IS} = 0.098). Populations from the same catchment or island were no more similar than populations from different islands, which suggests that occasional long-distance dispersal, both between and within islands, is the predominant influence on the population structure. This dispersal ability has contributed to the colonisation of most permanent streams on the Canary Islands by *M. aspersus*.

6.1 Introduction

Drainage networks can be viewed as 'habitat islands' surrounded by a 'sea' of land inhospitable to freshwater invertebrates. Colonisation of streams on oceanic islands is more problematic because of the dispersal barrier of the sea and, often, the scarcity of streams, resulting in aquatic taxa often being poorly represented on isolated islands (Wallace, 1880). The community present is strongly influenced by the dispersal abilities of the species in the archipelago species pool, their niche requirements and stochastic colonisation processes (Bunn and Hughes, 1997; Belyea and Lancaster, 1999). The archipelago species pool, in turn, is influenced by the chance dispersal of suitable species from a continental source pool (MacArthur and Wilson, 1967). The isolation and age of the Canary Islands, situated off the coast of Western Sahara, have resulted in a high degree of endemism in their flora and fauna. This is due to both the presence of taxa of Tertiary origin, which have become extinct elsewhere in their range, and post-colonisation speciation (Juan *et al.*, 2000).

Freshwater insects possess a wide variety of active and passive dispersal mechanisms (Williams and Hynes, 1976; Mackay, 1992). In-stream dispersal by active or passive drift, crawling, and swimming typically takes place at the reach scale but, over longer time scales, may allow colonisation of a whole stream system. Most freshwater insects can also disperse between water bodies as actively flying adults, allowing colonisation of other stream systems (Sheldon, 1984). Long distance dispersal of winged adults can additionally occur by passive drift in air currents (*e.g.* Clarke, 1903; Ashmole and Ashmole, 1988; Peck, 1994; Dobson, in press). The freshwater taxa occurring on the Canary Islands exhibit a range of dispersal abilities, mechanisms and distributions, from extremely localised to ubiquitous (Malmqvist *et al.*, 1995). Widespread species may have greater dispersal ability than species

with more restricted distributions, as range size and patch occupancy are often related to dispersal ability (Plague and McArthur, 1998). The relative lack of single-island endemic species within the Canarian freshwater fauna, compared to terrestrial invertebrates, is an indication that inter-island dispersal is substantial in most freshwater taxa (*e.g.* Peck, 1994). In the Coleoptera, for example, 4% of Dytiscidae are single-island endemics, compared to 54% of Carabidae (Machado, 1987, 1992; Alarie and Bilton, in press).

The dispersal ability of individual taxa determines the geographic scale of recruitment and, in combination with historical factors, the scale of population genetic differentiation (Slatkin, 1985a). Conversely, the degree of population differentiation observed at a particular scale can be used to infer the amount of dispersal (Bohonak, 1999a). Interpopulation dispersal reduces the genetic differentiation of populations that would otherwise occur through founder events, genetic drift and natural selection (Wright, 1943). Even ecologically trivial dispersal rates may have, over time, a significant impact on a species' population structure and biogeography (Williamson, 1981; Holt, 1993).

The island-like nature of stream habitats can potentially lead to genetic structuring of populations, which is likely to be enhanced by the distribution of the species across real islands. Several studies have used population genetic structure estimates to infer dispersal patterns from allozyme variation in stream invertebrates (Chapter 5). Some workers have found no evidence for isolation-by-distance and conclude that stochastic processes such as founder events and fluctuating population sizes are sufficient to explain the population genetic structure (*e.g.* Jackson and Resh, 1992; Bunn and Hughes, 1997; Byrne and Nichols, 1999). Others have demonstrated isolation-by-distance, suggesting an additional

influence of ongoing distance-dependent dispersal (*e.g.* Varvio-Aho and Pamilo, 1979; Dillon and Wethington, 1995; Hughes *et al.*, 1996).

In the present study, a survey of allozyme variation was made for *Mesophylax aspersus* Rambur, 1842 (Trichoptera: Limnephilidae) from five populations on three islands in the Canary archipelago. Three hypotheses about genetic variability, population structure and gene flow in *M. aspersus* were tested. Firstly, it was hypothesised that genetic variability would be lower than in continental populations/species Trichoptera, as island populations are likely to have undergone more marked bottlenecks and founder events (Gasperi *et al.*, 1991; Giller and Malmqvist, 1998), and as the sea may be a significant barrier to long-distance gene flow (Pashley *et al.*, 1985). The second hypothesis predicted that the species' genetic structure would be significant. This was because of the patchy nature of the stream habitat and the effect of the islands in isolating populations (*e.g.* Schug *at al.*, 1998; Thomas *et al.*, 1998), with populations nested by island and within island by watershed (*e.g.* Jackson and Resh, 1992; Hughes *et al.*, 1996; Bunn and Hughes, 1997). In addition, it was expected that interpopulation gene flow would be lower than in continental species, because of the greater difficulty of trans-oceanic dispersal (*e.g.* Mulvey *et al.*, 1988). The third and final hypothesis was that genetic differentiation of populations would increase with geographic distance regardless of island boundaries (*e.g.* Varvio-Aho and Pamilo, 1979; Dillon and Wethington, 1995).

6.2 Methods

6.2.1 Study species

Mesophylax aspersus has a circum-Mediterranean distribution, occurring from the Canary Islands to the Near East (*e.g.* Schmid, 1957; Botosaneanu, 1974; Dakki, 1987). The species is common on the western Canary Islands of Gran Canaria (Nybom, 1948, 1954; Nilsson *et al.*, 1998), Tenerife (Nybom, 1948; Malmqvist *et al.*, 1993), La Gomera (Nybom, 1954, and present study) and La Palma (present study). *M. aspersus* is found in most first and second order streams at altitudes of 200-2150m in a range of habitats including dense *laurisilva* woodland, open pine forest and agricultural land (Malmqvist *et al.*, 1995). Population densities were found to be as high as $2300m^{-2}$ (site T9, April 1998). An average abundance of $2.1m^{-2}$ (Tenerife, April 1991) was calculated by Malmqvist *et al.* (1993). *M. aspersus* was chosen as a representative widespread (on the Canary Islands) non-endemic species, to contrast with *Wormaldia tagananana* (Trichoptera: Philopotamidae), an endemic species with a restricted distribution (Chapter 7).

6.2.2 Localities and sampling

In April 1999, late-instar larvae of *Mesophylax aspersus* were collected from shallow pools in a set of five streams on three islands (Tenerife, La Gomera and La Palma), chosen to allow comparisons within and between catchments and islands (Figure 6.1). The study streams were P7, P10, T8, G1 and G4. They are located in Barranco Taburiente, La Palma, Barranco del Rio, La Palma, Barranco del Rio, Tenerife, and a tributary and the main channel at El Cedro, La Gomera, respectively (Section 2.2). In an attempt to sample from a single population, individuals were collected from 2-3 pools in a 5-10m stretch of stream (minimum sample size 24). Specimens were kept alive in insulated flasks of stream

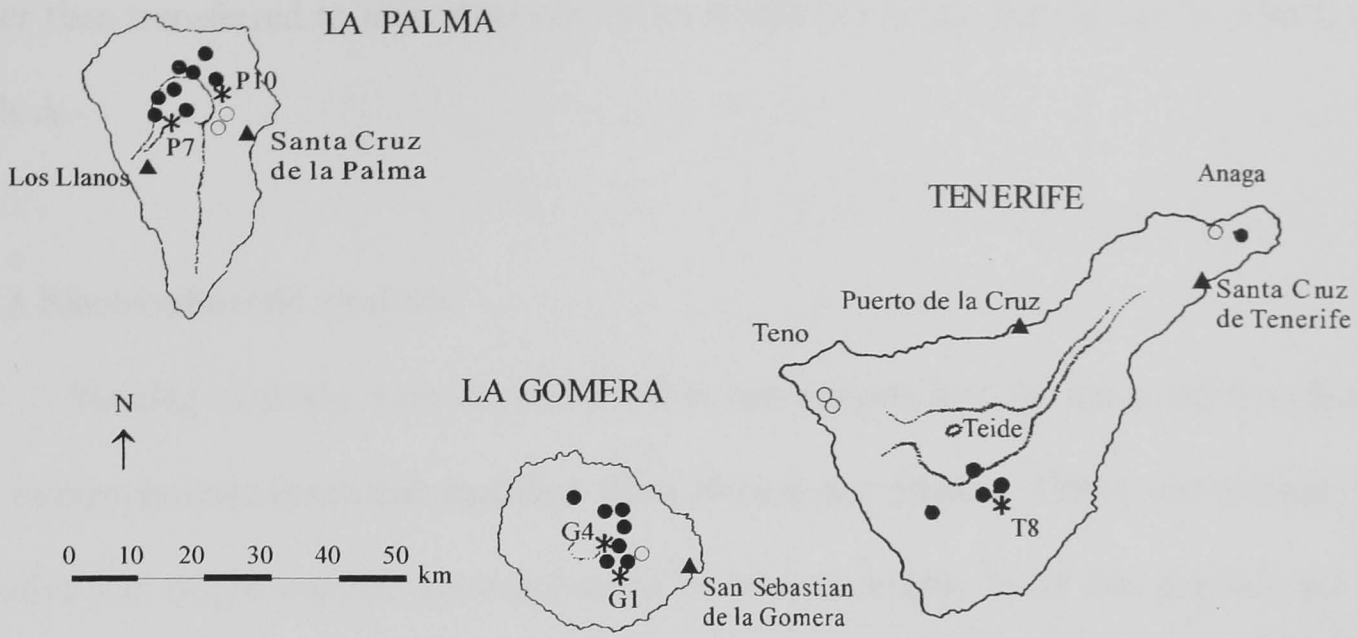


Figure 6.1 The distribution of *Mesophylax aspersus* in permanent streams on the western Canary Islands. ●: Species present; ❍: species absent; ✳: species present and population sampled; ▲: major town or city.

water then transferred to individual cryotubes within 2-3 hours, for storage at -196°C until analysis.

6.2.3 Electrophoretic analysis

Staining methods were devised for fourteen enzyme systems using cellulose acetate gel electrophoresis (protocol modified from Hebert and Beaton, 1991). From these, nine putative loci (eight enzyme systems) could be scored reliably in all five populations. The eight enzyme systems were EST, FUM, GPI, IDH, LAP (two loci), PEP C, PEP D and PGM. Full names and Enzyme Commission numbers (I.U.B.N.C., 1984) are listed in Appendix 6.3.

Larvae were removed from their cases and homogenised in 200µl of basic grinding buffer. Running buffers and stains were adapted from Richardson *et al.* (1986), Easteal and Boussy (1987), Hillis and Moritz (1990) and Hebert and Beaton (1991). Appendix 6.1 lists reagents used in cellulose acetate gel electrophoresis; Appendix 6.2 lists composition of buffer solutions used; Appendix 6.3 gives the specific staining methods developed for *Mesophylax aspersus*, with running buffer used, run time and incubation time. Run times varied from 20-40min and incubation times from 5-40min. Rat liver tissue (adult male Sprague-Dewley rats) was run in one lane on each gel as a positive control. Loci and alleles were labelled numerically and alphabetically respectively, in ascending order from the least to the most mobile.

6.2.4 Statistical analysis

The data were summarised as allele frequencies at each locus in each population with the BIOSYS-1 package (Swofford and Selander, 1989). As measures of genetic

variability, the mean number of alleles (*MNA*) per locus, the percentage of polymorphic loci (*P*) at the 95% level and expected heterozygosity (*H*) (Nei's 1978 unbiased estimate) were calculated with BIOSYS-1. Population differentiation and structure was investigated with *F* statistics (Wright, 1951, 1969) estimated by the formulae of Weir and Cockerham (1984) with the GENETIX package (Université de Montpellier II, 1999). Standard deviations of the multilocus *F* statistic estimates were obtained by jack-knifing over loci. Comparing the observed means to the outcomes generated from permutation tests estimated significance: to test F_{IS}, alleles were randomised within populations; to test F_{ST}, individual genotypes were randomly allocated to populations. A sequential Bonferroni correction for the analysis of multiple tests was used (Rice, 1989), calculated by hand. Multilocus F_{ST} was calculated for each pair of sites. Pair-wise site comparisons were also performed using Rogers' (1972) genetic distance, calculated with BIOSYS-1. Significance was estimated by comparing the observed distances with a null distribution generated by recalculating the distance matrix after 1000 random reassignments of individuals to sites. The models upon which *F* statistics and genetic distance measures are based make a number of biologically unrealistic assumptions (Section 5.1.6), therefore care has to be taken not to over-interpret results.

A dendrogram showing the relationships between the sites was constructed by the distance Wagner (Farris, 1972) procedure with BIOSYS-1, using a matrix of Rogers' genetic distance (distance Wagner requires a metric distance measure which satisfies the triangular inequality). The dendrogram was rooted at the midpoint of the longest path. Default criterion II for the sequence of addition of sites to the developing tree was used. The criterion for selecting partial networks to be saved for the next step of the algorithm was the default, Prager and Wilson's (1976) *F* value. In order to determine whether the population genetic structure was consistent across loci, the Rogers' distance matrix and

distance Wagner tree-building procedure were repeated for the EST enzyme system, as this gave the most variable locus scored at all five sites.

Multilocus F_{ST}, and Rogers' genetic distance, for each pair of sites were plotted against geographical distance between sites and minimum inter-island distances, both directly and with log transformations. Distances were defined as the shortest measurements on the map, in the first instance between sites, and in the second instance the shortest sea crossing between islands (Figure 6.2). Use of the distance between sites assumes that the genetic relationships of populations reflect current dispersal, whilst use of inter-island distance assumes a reflection of current *and* historic dispersal, given that in the past suitable stream habitats are likely to have occurred at higher densities on the islands (Section 1.4). The relationships between the genetic and geographic distances were tested formally with Mantel tests (Mantel, 1967; Manly, 1986; Smouse *et al.*, 1986) in the GENETIX package.

6.3 Results

6.3.1 Genetic variability measures

All loci, with the exception of FUM, were polymorphic in at least one population, and EST, LAP-1 and PEP C were polymorphic in every population (Table 6.1). There was large variation in allele frequencies between populations, and at only two loci was the most common allele constant across populations. However there was only one site-specific (allele B of FUM locus at P7) and no island-specific alleles. Populations at the five sites showed different amounts of variability, with the Tenerife sample showing particularly little: *MNA*, *P* and mean *H* were all lowest at T8, however *H* was not significantly lower.

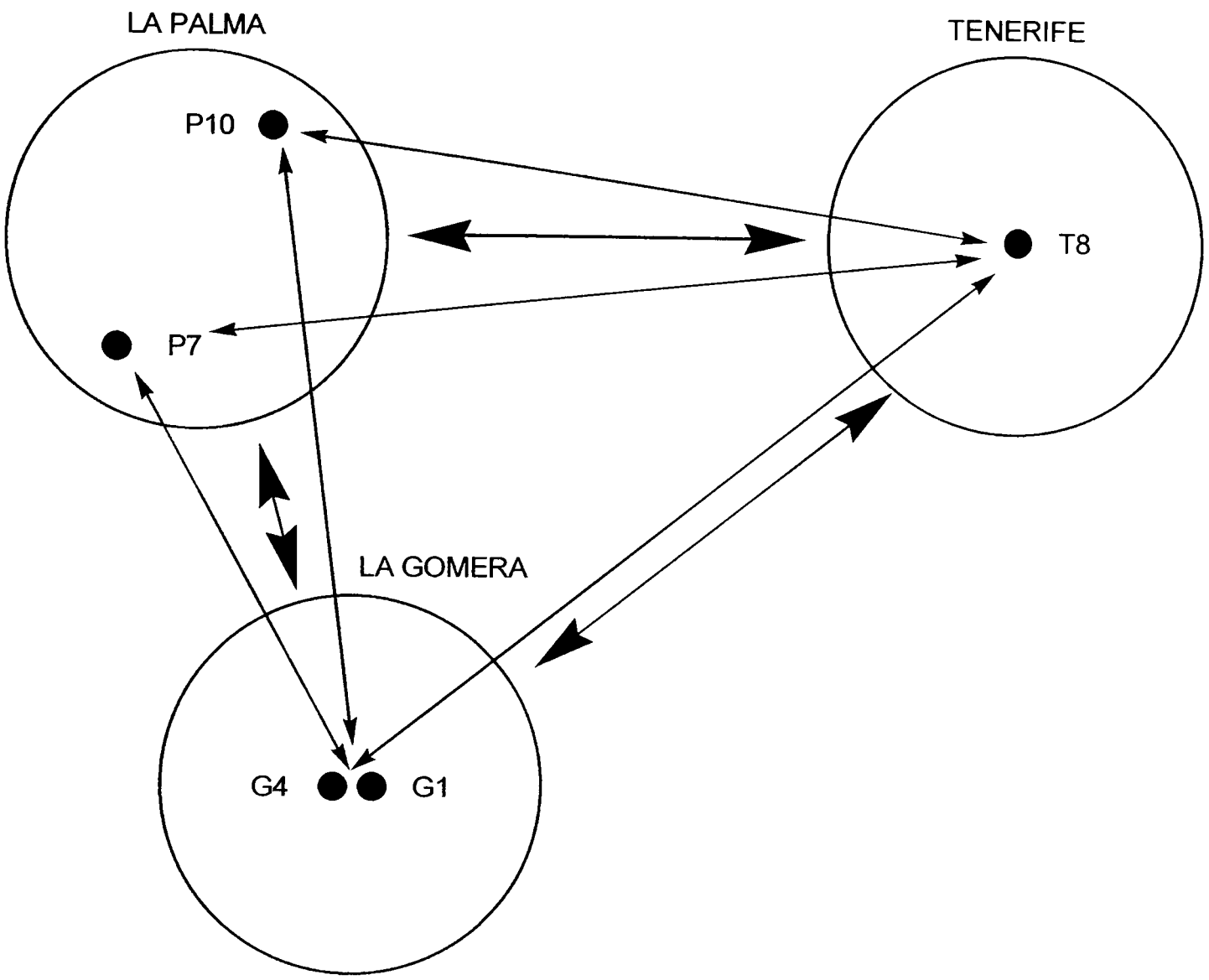


Figure 6.2 Diagrammatic representations of minimum inter-island and inter-site distances. The close proximity of sites G1 and G4 means that pair-wise distances involving these sites and those on other islands will effectively be the same. Likewise, inter-island distances will be replicated where there is more than one site on an island.

Locus	Allele	P7	P10	T8	G1	G4
EST	(N)	55	36	21	40	32
	A	0.064	0.194	0.024	0.013	0.047
	B	0.273	0.403	0.238	0.538	0.594
	C	0.445	0.306	0.5	0.3	0.266
	D	0.218	0.097	0.238	0.15	0.094
FUM	(N)	48	37	24	40	20
	A	0.979	1	1	1	1
	B	0.021	0	0	0	0
GPI	(N)	53	40	20	40	31
	A	0.66	0.1	1	0.325	0.355
	B	0.34	0.9	0	0.675	0.645
IDH	(N)	46	28	18	35	32
	A	1	0.982	1	0.843	1
	B	0	0.018	0	0.157	0
LAP-1	(N)	54	34	17	37	32
	A	0.704	0.559	0.088	0.446	0.531
	B	0.296	0.441	0.912	0.554	0.469
LAP-2	(N)	52	29	15	32	28
	A	0.077	0.379	0	0	0.714
	B	0.923	0.621	1	1	0.286
PEP C	(N)	56	37	22	39	28
	A	0.054	0	0.023	0.013	0
	B	0.946	0.878	0.841	0.91	0.964
	C	0	0.122	0.136	0.077	0.036
PEP D	(N)	30	38	22	40	30
	A	1	0.289	1	0.85	0.567
	B	0	0.711	0	0.15	0.433
PGM	(N)	50	35	19	28	23
	A	0.14	0.257	0	0.018	0.109
	B	0.86	0.629	1	0.714	0.196
	C	0	0.114	0	0.268	0.696
MNA		2	2.222	1.667	2.222	2.111
S.D.		0.87	0.83	1.1	0.97	0.93
P (95%)		66.67	77.78	33.33	77.78	66.67
H		0.230	0.336	0.119	0.293	0.328
S.D. (*H*)		0.236	0.239	0.217	0.212	0.234

Table 6.1 Allele frequencies in five *Mesophylax aspersus* populations. Alleles labelled A to D at each locus. (N): the number of individuals for which the locus was scored; *MNA*: mean number of alleles scored per locus; *P*: percentage of polymorphic loci at 95% criterion; and *H*: unbiased estimate of expected heterozygosity.

6.3.2 Population differentiation and structure

A summary of F statistics is provided in Table 6.2. F_{IS} by locus by population was found to be very variable. Significance is indicated in Table 6.2, reflecting the number of records available as well as the calculated value of F_{IS}. P7 and G1 showed a non-significant excess of heterozygotes across all loci whilst P10, T8 and G4 showed a deficiency (significant for P10 only). Considering F_{IS} for individual loci by population, LAP-1 had a particular excess of heterozygotes, significant in all populations except T8, whilst for PEP D there was an excess of heterozygotes at site G1 but a significant deficiency at P10 and G4 (with no heterozygotes at all at G4).

The multilocus estimates of F_{IS} and F_{IT} were significantly positive. The multilocus F_{ST} was 0.250, which implies substantial population structuring ($p < 0.001$). All the pair-wise genetic distances (both F_{ST} and Rogers' distance) were significant ($p < 0.05$ after Dunn-Šidak correction for multiple significance tests) (Table 6.3). The most distant pair of sites was T8-G4, and the closest P7-G1 (Table 6.3). The distance Wagner dendrogram had a cophenetic correlation coefficient (Sneath and Sokal, 1973) of 0.966. It was redrawn as a network to clarify the site and island relationships (Figure 6.3). The branching order and relative branch lengths showed that sites within an island were not more similar than sites on different islands. The distance Wagner network produced for the EST locus (not shown) had a different topology, with G1 and G4 grouped together (cophenetic correlation coefficient = 0.902).

6.3.3 Genetic distance and geographical isolation

Regressions of pair-wise F_{ST} and Rogers' genetic distance against geographic distances were non-significant (not shown). Mantel tests on each pair of matrices confirmed

	F_{IS} by Locus by Population					F statistics by Locus		
Locus	P7	P10	T8	G1	G4	F_{IS}	F_{IT}	F_{ST}
EST	*0.338	*0.417	0.438	0.055	0.196	0.307	0.338	0.045
FUM	-0.011					-0.006	-0.002	0.004
GPI	-0.338	0.179		0.214	0.030	-0.039	0.333	0.358
IDH		0.000		-0.172		-0.155	-0.008	0.127
LAP-1	*-0.413	*-0.662	-0.067	*-0.800	*-0.750	-0.618	-0.382	0.145
LAP-2	0.917	*0.858			-0.032	0.398	0.671	0.453
PEP C	-0.048		-0.143	*-0.074		-0.094	-0.065	0.026
PEP D		*0.875		*-0.164	*1.000	0.664	0.806	0.422
PGM	-0.153	0.072		0.132	*0.836	0.277	0.496	0.302
All loci	-0.151	*0.264	0.110	-0.172	0.166	***0.098	***0.323	***0.250
Resampling mean						0.099	0.323	0.247
S.E.						0.179	0.152	0.069

Table 6.2 *F* statistics for five *Mesophylax aspersus* populations. F_{IS} is calculated over all alleles at polymorphic loci in each population, and F statistics for each locus over all populations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Blank cells indicate fixed homozygous loci.

Site	P7	P10	T8	G1	G4
P7	0	0.280	0.216	0.118	0.330
P10	0.250	0	0.426	0.165	0.142
T8	0.152	0.342	0	0.259	0.460
G1	0.169	0.203	0.216	0	0.224
G4	0.286	0.188	0.380	0.207	0

Table 6.3 Interpopulation genetic distances for *Mesophylax aspersus*. All $p < 0.05$. Above the diagonal: θ, an estimator of F_{ST} (Weir and Cockerham, 1984); below the diagonal: Rogers' genetic distance (1972).

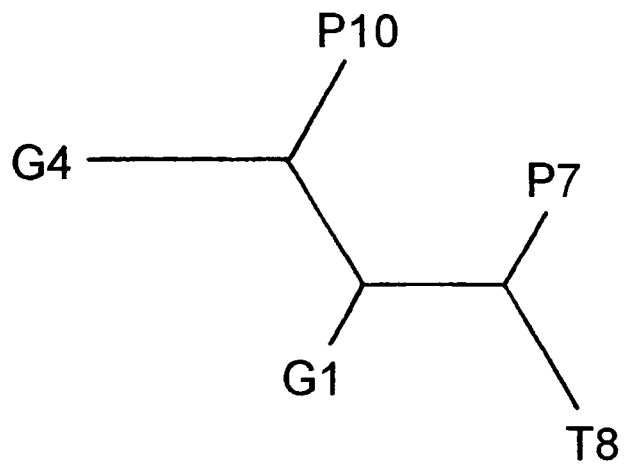


Figure 6.3 A distance Wagner network of genetic distances between populations of *Mesophylax aspersus*. Rogers' (1972) genetic distance was used in the distance Wagner procedure; dendrogram redrawn as an unrooted network.

that there was no significant pattern of isolation-by-distance (*Z* statistic did not differ significantly from the null distribution).

6.4 Discussion

6.4.1 Genetic variability compared to continental species

Levels of genetic variability in *Mesophylax aspersus* were generally high, except at T8. The mean H and P were higher than any previously recorded in Trichoptera (Plague and MacArthur, 1988; Jackson and Resh, 1992; Guinand, 1994) (Table 6.4), falsifying the first stated hypothesis that genetic variation would be lower in island populations of *M. aspersus* than in continental species studies. MNA of *M. aspersus* was more typical of Trichoptera, though still higher than previously observed, at all sites but T8. The lack of site- or island-specific alleles suggests that the populations did not originate independently.

The most likely explanation of the high genetic variability in *M. aspersus* is occasional interpopulation dispersal of individuals between populations with different genetic composition (Slatkin, 1985a; Leberg, 1992), despite their geographic isolation and the dispersal barrier of the sea. If populations are of reasonable size and longevity then genetic variability can accumulate. Balancing selection and temporal and spatial variation in selection pressures may maintain some of the genetic diversity. The genetic variability estimates are likely to be inflated by the lack of monomorphic loci in the data set; however further work showed that of an additional ten loci none were monomorphic. It would be of interest to make a comparable study of continental populations of *M. aspersus*, providing a direct test of the effect of distribution across oceanic islands on genetic diversity.

Species	Pops.	Scale (km)	*N*	Loci	*MNA*	*P* (%)	H_{exp}	F_{ST}	Ref.[a]
Cheumato-psyche edista	2	11	16-63	19	1.6	26%	0.07 (0.04-0.1)	NA	1
C. pasella	2	11	16-63	19	1.85	36%	0.15 (0.105-0.195)	NA	1
C. pettiti	2	11	16-63	19	1.4	12%	0.035 (0.02-0.5)	NA	1
C. pinacea	2	11	16-63	19	1.95	42%	0.15 (0.11-0.19)	NA	1
C. richardsoni	2	11	16-63	19	1.4	11%	0.035 (0.015-0.055)	NA	1
Helicopsyche borealis sp. A	9 9 12	5-20 <200 <2000	30-34 30-34 13-34	17	1.55	21.19% (11.8-29.4)	0.09 (0.059-0.118)	0.008-0.034 0.425 0.524	2
Helicopsyche borealis sp. B	1	NA	34	17	1.8	29.4%	0.114	NA	2
Helicopsyche borealis sp. C	2	7	2-26	17	1.15	11.8 (5.9-17.7)	0.056 (0.039-0.073)	NA	2
Helicopsyche borealis sp. D	1	NA	3	17	1.1	11.8%	0.039	NA	2
Hydropsyche exocellata	2	25	17-43	12	1.538	62.5% (58.3-66.7)	0.133[b] (0.131-0.134)[b]	0.015	3
Tasiagma ciliata	12 10 10	<0.3 <6 <25	23-109 4 32-	4 4 4	5[c] 5[c] 5[c]	100%[c]	NA NA NA	0.007-0.127 0.003-0.013 0.012	4

[a] References: (1) Plague and MacArthur (1988); (2) Jackson and Resh (1992); (3) Guinand (1994); (4) Hughes *et al.* (1998).

[b] H_{obs}.

[c] Polymorphic loci with particularly high *MNA* were selected by Hughes, J.M. *et al.* (1998).

Table 6.4 Genetic variability data from allozyme studies on Trichoptera in the literature. Number of populations surveyed (Pops.), geographic scale of sampling (Scale), number of specimens screened per population (*N*) and number of loci scored (Loci) are given. Mean number of alleles per locus (*MNA*), mean and range of the percentage of polymorphic loci (*P*) at 95% criterion and mean and range of the expected heterozygosity (H_{exp}) are calculated.

The lower heterozygosity found in other studies of Trichoptera (Table 6.4) may also be due in part to the sampling methods employed. Attracting adults to a light trap may inadvertently sample individuals from more than one population (*e.g.* Plague and McArthur, 1998), whilst larvae collected from a small area of a stream may represent only one or a few sibling groups (*e.g.* Jackson and Resh, 1992; Guinand, 1994; Bunn and Hughes, 1997). Both these scenarios would produce a distorted picture of genetic variation at the population level. In the case of sampling across populations, homozygosity is overestimated due to the Wahlund effect (Lees and Ward, 1987; Hartl and Clark, 1997; Arnaud *et al.*, 1999).

6.4.2 Population structure: genetic and geographic isolation

Mesophylax aspersus has substantial population structuring on the Canary Islands, as predicted. F_{IS} was significantly positive overall but varied in sign from locus to locus. Possible explanations are that null alleles confounded the scoring of gels, or that selection is acting upon some loci (*e.g.* against homozygotes at LAP-1), whilst others may be subject to genetic drift alone. The Wahlund effect may have produced significant F_{IS} in the study on *Cheumatopsyche* by Plague and McArthur (1998) but it is not likely to have operated alone in the present study, as heterozygote excess as well as deficiency was found.

The hypothesis of a hierarchical population structure in *M. aspersus* was not supported as population subdivision was as significant within as between islands, and same-island pairs had genetic distances in the mid-range of the pair-wise distances. The patchy nature of suitable stream habitat may make dispersal between streams on the same island as unlikely as dispersal over the sea. In contrast, Jackson and Resh (1992) found that genetic variation in *Helicopsyche* was hierarchically structured, with smaller differences in allele

frequencies observed among sites within a stream and larger differences between catchments and regions. The differentiation of G1 and G4, only 200m apart, is a striking result inviting further sampling on a scale of tens to hundreds of metres within the catchment, to ascertain the scale of the panmictic unit and the extent of the stochastic effect of recruitment (*e.g.* Giles *et al.*, 1998; Knoll and Rowell-Rahier, 1998; Hughes *et al.*, 1998).

Similar values of multilocus F_{ST} are reported for *M. aspersus* and the continental species, when populations are separated by comparable geographic distances: F_{ST} = 0.425 over 200km in *Helicopsyche borealis* (Jackson and Resh, 1992); F_{ST} = 0.015 over 25km in *Hydropsyche exocellata* (Guinand, 1994). Thus, the prediction that interpopulation gene flow would be lower in *M. aspersus* was not supported. However single locus F_{ST} in *M. aspersus* varied by 2 orders of magnitude, and this heterogeneity means that the multilocus estimator should be interpreted with caution (Felsenstein, 1982; Guinand, 1994). Heterogeneity of F_{ST} across loci suggests that selection is acting on some loci but not others (Barbujani, 1985). The influence of selection is also suggested by the difference between distance Wagner networks produced for all loci and for EST alone. Interpopulation distances are the product of a combination of selection, drift, dispersal and population history; of these, selection may vary from locus to locus. Balancing selection and spatial and temporal variation in selection pressures may all increase genetic diversity in the species.

The final hypothesis was that F_{ST} would increase with geographic distance, whether within or between islands. This was not supported. This implies that streams will not necessarily be colonised by the nearest neighbouring population. Dispersal between sites in close proximity could be prevented by: prevailing wind direction; topography, particularly

when streams are in deep gorges (as are P7, P10, and T8); dense forest (as surrounds P10, G1, and G4); and low stream density (as on Tenerife). Passive dispersal over longer distances could occur if an airborne insect became caught in a wind current, as studies of insect fallout on the snowfields of Mount Teide, Tenerife (Ashmole and Ashmole, 1988), on ships and over the sea (Clarke, 1903) have demonstrated. The process of dispersal could be studied further by investigating the effects of putative dispersal barriers on small-scale genetic differentiation.

A number of similar studies have failed to find isolation-by-distance (e.g. Jackson and Resh, 1992; Bunn and Hughes, 1997; Byrne and Nichols, 1999), and the stochastic effect of recruitment, random dispersal, population history and environmental structure are invoked. However the influences of current gene flow (dispersal) and historic gene flow (population history) often cannot be distinguished (Slatkin, 1985a; Bossart and Prowell, 1998), particularly when isolation-by-distance is not found (Slatkin and Maddison, 1990). In this case, division of the species' range into an archipelago of islands does not determine its genetic structure, and genetic variability within populations suggests that the stochastic effect of recruitment is also not the cause of the population structure.

6.4.3 Genetic differentiation and dispersal

Bohonak (1999a) found that there is a robust relationship between population structure and dispersal ability: genetic distance estimates are informative and patterns of dispersal do make a measurable contribution to observed population genetic structure in the majority of comparisons (*e.g.* Waples, 1987). This study makes use of this paradigm to infer dispersal ability from genetic differentiation in order to investigate the relationship between dispersal ability and distribution of *Mesophylax aspersus*. It is likely that *M. aspersus* is the

strongest flier of the Canarian trichopteran fauna (Göthberg, 1973; Svensson, 1974; Coutant, 1982), and that adult flight is the principal mechanism of dispersal in Trichoptera (Bunn and Hughes, 1997). It is concluded that a small amount of distance-independent dispersal of individuals between populations occurs, which has allowed *M. aspersus* to colonise almost all the permanent streams in the archipelago. Whilst the paucity of streams on the Canary Islands leaves freshwater fauna isolated in an otherwise arid environment, populations of *M. aspersus* appear to be large and persistent enough, and receive enough genetically distinct immigrants, to maintain high levels of genetic variability within them.

Chapter 7

Population Structure and Dispersal of

Wormaldia tagananana

(Trichoptera: Philopotamidae)

Population Structure and Dispersal of *Wormaldia taganananana* (Trichoptera: Philopotamidae)

SUMMARY

Population genetic structure of the Canarian endemic caddisfly *Wormaldia taganananana* (Trichoptera: Philopotamidae) was investigated by studying allozyme variation at eleven putative loci in five of the eight extant populations on Tenerife and La Gomera. Genetic variability, population structure and gene flow were compared with those found for Canarian populations of the more widespread species *Mesophylax aspersus* (Trichoptera: Limnephilidae) and with data in the literature. This enabled an assessment to be made of the relationship between distributional range size and population genetic variation and structure. Genetic variability was lower than that recorded for *M. aspersus*, *e.g.* mean heterozygosity was 0.025-0.186 (0.119-0.336 in *M. aspersus*), but broadly similar to that found in previous studies of more widespread, continental species: small range size is thus not accompanied by low genetic variation in *W. taganananana*. Significant population structure was observed (overall F_{ST} = 0.387), greater even than that found for *M. aspersus*, and amongst the highest reported for lotic caddis to date. There was also highly significant within-population structuring (overall F_{IS} = 0.675), perhaps resulting from larvae within a reach being the product of only a few matings. A non-significant trend towards isolation-by-distance was observed, with greater gene flow between populations in close proximity than between more distant sites. Several site- and island-specific alleles were recorded, providing further evidence for the relative isolation of *W. taganananana* populations. This suggests that dispersal is more limited, and distance-dependent, in *W. taganananana* than in *M. aspersus*. The genetic evidence provides support to the hypothesis that the restricted range of *W. taganananana* is due at least in part to limited dispersal ability.

7.1 Introduction

Chapters 5 and 6 described the use of allozymes in ecological and biogeographical studies, and a study of genetic differentiation in *Mesophylax aspersus* (Trichoptera: Limnephilidae), a non-endemic species widespread on the Canary Islands. In the present chapter, *M. aspersus* is contrasted with the population structure and dispersal patterns of a caddisfly species, *Wormaldia tagananana* (Enderlein, 1929) (Trichoptera: Philopotamidae), endemic to the Canary Islands and restricted in its distribution to only eight streams on La Gomera and Tenerife, where it is locally abundant.

The comparative analysis of population genetic structure and gene flow can be used to determine the relative dispersal ability of taxa (Section 5.2.5). The dispersal ability of a species determines the geographical scale of recruitment and, in combination with historical factors, the scale of population differentiation by counter-acting genetic drift (Wright, 1943; Slatkin, 1985a). Population differentiation and dispersal ability are negatively correlated (Waples, 1987; Bohonak, 1999b). Bohonak (1999a) reviewed the allozyme studies literature for groups of species which were 'phylogenetically, geographically and demographically comparable', and concluded that this relationship between dispersal ability and genetic differentiation was robust.

The community present in a stream is dependent upon the dispersal abilities and niche requirements of species in the regional pool, and on stochastic colonisation processes (Bunn and Hughes, 1997; Belyea and Lancaster, 1999; Pulliam, 2000). The different distributions of *M. aspersus* and *W. tagananana* suggest that the two species differ in these characteristics. There is generally assumed to be a positive relationship between dispersal ability and geographic range size, suggested by observations on a variety of plant and

animal groups (Gaston, 1994; Maurer, 1999; Gaston and Blackburn, 2000), and range size and patch occupancy have been related to dispersal ability as inferred from genetic analysis (Plague and MacArthur, 1998). It is likely that caddis larvae disperse over very short distances up- and downstream, and that the adults are generally active dispersers, but may have a short dispersal range (Sheldon, 1984; Mackay, 1992; Bunn and Hughes, 1997). Genetic analysis offers an indirect method of studying rare long-distance inter-population dispersal by flying adults, impossible to observe directly (Bilton *et al.*, in press).

Allozyme variation in five populations of *W. tagananana* was surveyed to test three hypotheses about the genetic variability, population structure and dispersal of this species compared to that of *M. aspersus.* If the restricted distribution of *W. tagananana* is due to little dispersal, genetic variability both within and across populations was predicted to be low, particularly when compared to that found for *M. aspersus* (Chapter 6). This is because populations are more susceptible to bottlenecks and loss of diversity through genetic drift when interpopulation dispersal is low (Haydon *et al.,* 1993). Secondly, it was hypothesised that significant population structure would be found, and that it would be more significant than that found for *M. aspersus*, due to reduced interpopulation dispersal in *W. tagananana* (Slatkin, 1987; Liebherr, 1988). Thirdly, it was hypothesised that interpopulation gene flow would be lower in *W. tagananana*, and would be distance-dependent, producing a pattern of isolation-by-distance regardless of isolation boundaries (*e.g.* Varvio-Aho and Pamilo, 1979; Mulvey *et al.*, 1988; Dillon and Wethington, 1995).

7.2 Methods

7.2.1 Study species

Wormaldia taganananana was recorded only at Masca (Teno region) and Ijuana (Anaga region) on Tenerife, and from the El Cedro stream system and three other streams on La Gomera, eight streams. The species has previously been found at a few other sites on Tenerife and La Gomera (Nybom, 1948, 1954; Botosaneanu, 1981), but it may have suffered local extinction due to habitat destruction (water loss). The species is the only member of the Philopotamidae on the islands. It is found in both *laurisilva* and deforested catchments, in first and second order streams at altitudes of 350-1020m. Population densities were found to be as high as $592m^{-2}$ (site G1, April 1998). An average abundance range of $0.4\text{-}12m^{-2}$ (Tenerife, April 1991) was calculated by Malmqvist *et al.* (1993). The range size and distribution of *W. taganananana* contrasts with that of *Mesophylax aspersus* (Trichoptera: Limnephilidae) (Chapter 6), and is typical of a number of Canarian species.

7.2.2 Localities and sampling

In April 1999, late-instar larvae of *Wormaldia taganananana* were collected from shallow pools in five streams selected for their abundance of this species and to allow comparisons within and between islands and catchments (Figure 7.1). The study streams were G1, G4, T2, T3 and T4. They are a tributary and the main channel at El Cedro (La Gomera), the Ijuana stream (Anaga, Tenerife), and the main channel and a tributary at Masca (Teno, Tenerife), respectively. In an attempt to sample from a single population, individuals were collected from 2-3 pools in a 5-10m stretch of stream. Sample size was 8-42 per site, comparable to that for *Mesophylax aspersus*, important for the validity of inter-species comparisons (Hartl and Clark, 1997). Specimens were kept alive in insulated flasks

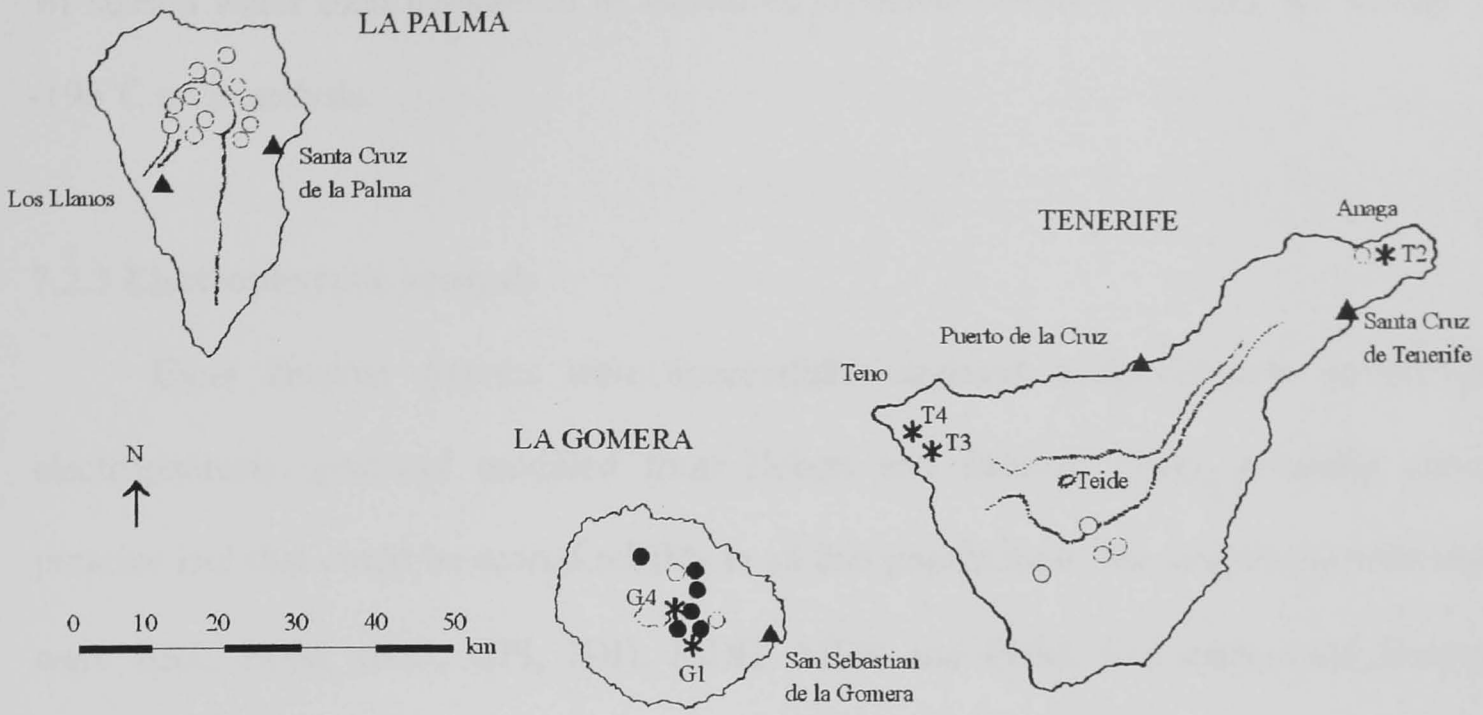


Figure 7.1 The distribution of *Wormaldia tagananana* in permanent streams on the western Canary Islands. ●: Species present; ○: species absent; ✳: species present and population sampled; ▲: major town or city.

of stream water then transferred to individual cryotubes within 2-3 hours, for storage at -196°C until analysis.

7.2.3 Electrophoretic analysis

Eight enzyme systems were successfully screened using cellulose acetate gel electrophoresis (protocol modified from Hebert and Beaton, 1991), revealing eleven putative loci that could be scored reliably in all five populations. The enzyme systems used were EST, FUM, αGP, GPI, IDH, MDH, MEN and PGM. Full names and Enzyme Commission numbers (I.U.B.N.C., 1984) are listed in Appendix 7.1.

Larvae were removed from their cases and homogenised. In an effort to refine the electrophoretic method, a variety of buffer solutions was utilised. For the Tenerife samples, 200µl of basic grinding buffer was used. Samples from La Gomera were homogenised in 100µl of grinding buffer, and four different grinding buffers were tried on individuals from the G1 sample. These were the basic homogenising buffer, basic minus mercaptoethanol, PTP homogenising buffer and the Peakall and Beattie (1991) homogenising buffer. The Peakall and Beattie buffer was used for the remainder of the La Gomera samples. Running buffers and stains were adapted from Richardson *et al.* (1986), Easteal and Boussy (1987), Hillis and Moritz (1990) and Hebert and Beaton (1991). Appendix 6.1 lists reagents used in cellulose acetate gel electrophoresis; Appendix 6.2 lists composition of buffer solutions used; Appendix 7.1 gives the specific staining methods developed for *Wormaldia taganananа*, with running buffer used, run time and incubation time. Run times varied from 20-40min and incubation times from 5-40min. Rat liver tissue (adult male Sprague-Dewley rats) was run in one lane on each gel as a positive control. Loci and alleles were labelled numerically and alphabetically respectively, in ascending order from the least to the most mobile.

7.2.4 Statistical analysis

The data were summarised as allele frequencies at each locus in each population with the GENETIX package (Université de Montpellier II, 1999). As measures of genetic variability, the mean number of alleles (*MNA*) per locus, the percentage of polymorphic loci (*P*) at the 95% level and expected heterozygosity (*H*) (Nei's (1978) unbiased estimate) were then calculated. One-tailed Student's *t* tests, assuming equal variance, were used to test the hypothesis that genetic variability is lower in *Wormaldia taganananа* than in *Mesophylax aspersus*.

Population differentiation and structure were investigated with *F* statistics (Wright, 1951, 1969) estimated by the formulae of Weir and Cockerham (1984) with GENETIX. Standard deviations of the multilocus *F* statistic estimates were obtained by jack-knifing over loci. Comparing the observed means to the outcomes generated from permutation tests estimated significance: to test F_{IS}, alleles were randomised within populations; to test F_{ST}, individual genotypes were randomly allocated to populations. A sequential Bonferroni correction for the analysis of multiple tests was used (Rice, 1989), calculated by hand. Multilocus F_{ST} was calculated for each pair of sites. Pair-wise site comparisons were also performed using Rogers' (1972) genetic distance, calculated with GENETIX. Significance was estimated by comparing the observed distances with a null distribution generated by recalculating the distance matrix after 1000 random reassignments of individuals to sites.

A dendrogram showing the relationships between the sites was constructed by the distance Wagner (Farris, 1972) procedure with BIOSYS-1 (Swofford and Selander, 1981, 1989), using a matrix of Rogers' genetic distance (distance Wagner requires a metric distance measure which satisfies the triangular inequality). The dendrogram was rooted at the midpoint of the longest path. The default criterion II for the sequence of addition of

sites to the developing tree was used. The criterion for selecting partial networks to be saved for the next step of the algorithm was the default, Prager and Wilson's (1976) *F* value.

Multilocus F_{ST}, and Rogers' genetic distance, for each pair of sites were plotted against geographical distance between sites and minimum inter-island distances (Section 6.2.4, Figure 6.2), both directly and with log transformations. Distances were defined as the shortest measurements on the map. The relationships between the genetic and geographic distances were tested formally with Mantel tests (Mantel, 1967; Manly, 1986; Smouse *et al.*, 1986) in the GENETIX package.

7.3 Results

7.3.1 Genetic variability measures

The most variable loci were FUM and MDH, which were polymorphic in all the populations in which they were scored (Table 7.1). Of the eleven loci, four appeared to be monomorphic. Allele frequencies varied between sites, but to a lesser degree than in *Mesophylax aspersus*: at four of the polymorphic loci, the most common allele was constant across populations. MNA, P and H were each very similar across sites T2, T3, G1 and G4. The population at T4 showed lower genetic variability by all three measures, most likely due to the small sample size. MNA, P and H were all significantly lower in *Wormaldia taganananа* than *M. aspersus* (Student's *t* tests, all $p < 0.02$). Two site-specific alleles (IDH-1 allele B at T3 and MDH allele C at G1) and one island-specific allele (IDH-2 allele A on Tenerife) were found, compared to only one for *M. aspersus*.

Locus	Allele	T2	T3	T4	G1	G4
EST-1	(N)	11	0	0	36	30
	A	0.909			0.917	0.967
	B	0.091			0.083	0.033
EST-2	(N)	19	26	8	36	32
	A	1	1	1	1	1
FUM	(N)	20	4	0	20	30
	A	0.55	0.375		0.6	0.433
	B	0.45	0.625		0.4	0.567
αGP-1	(N)	24	16	3	38	32
	A	1	1	1	1	1
αGP-2	(N)	0	2	0	30	25
	A		1		1	1
GPI	(N)	24	16	0	33	31
	A	0	0.031		0.273	0.339
	B	1	0.969		0.727	0.661
IDH-1	(N)	33	24	8	39	32
	A	1	0.667	1	1	1
	B	0	0.333	0	0	0
IDH-2	(N)	33	28	8	39	32
	A	0.667	0.179	0	0	0
	B	0.333	0.821	1	1	1
MDH	(N)	23	12	0	36	32
	A	0.022	0.792		0.375	0.672
	B	0.978	0.208		0.292	0.328
	C	0	0		0.333	0
MEN	(N)	42	20	8	38	32
	A	1	1	0.938	0.803	0.781
	B	0	0	0.063	0.197	0.219
PGM	(N)	1	8	0	29	31
	A	1	1		1	1
MNA		1.4	1.5	1.2	1.546	1.455
S.D.		0.52	0.53	0.45	0.69	0.52
P (95%)		30.0	40.0	20.0	45.45	36.36
H		0.118	0.170	0.025	0.186	0.165
S.D. (*H*)		0.199	0.215	0.056	0.246	0.220

Table 7.1 Allele frequencies in five *Wormaldia taganananа* populations. Alleles labelled A to C at each locus. (N): number of specimens for which the locus scored; *MNA*: mean number of alleles scored per locus; *P*: percentage of polymorphic loci at 95% criterion; *H*: unbiased estimate of expected heterozygosity.

7.3.2 Population differentiation and structure

A summary of *F* statistics is provided in Table 7.2. F_{IS} was variable, including both significant positive and significant negative values for individual loci in individual populations. A significant deficiency of heterozygotes was recorded for FUM, GPI, IDH-1, IDH-2 and MEN, in at least one population. A significant excess of heterozygotes was recorded for GPI and MDH, in one population each. Overall, sites T2 and G1 showed a significant deficiency of heterozygotes. The multilocus estimates of F_{IS} and F_{IT} were significantly positive ($p < 0.001$). The multilocus estimate of F_{ST} was also significant, 0.387 ($p < 0.001$), which implies that *Wormaldia tagananana* has very substantial population structuring.

Seven of the ten pair-wise F_{ST} values were significant ($p < 0.05$, after Dunn-Šidak correction for multiple significance tests) (all but T3-T4, T4-G1 and T4-G4) (Table 7.3). The most distant pair of sites was T2-T4/T2-G4 and the closest T4-G4/G1-G4, depending on the measure used (Table 7.3). The distance Wagner dendrogram had a cophenetic correlation coefficient (Sneath and Sokal, 1973) of 0.981. It was redrawn as a network to clarify the site and island relationships (Figure 7.2). The branching order and relative branch lengths showed that populations at G1 and G4 are genetically similar, and are grouped with the anomalous population at T4. T2 and T3 are not grouped, which may reflect their geographical remoteness from one another at opposite ends of Tenerife with little suitable habitat between. The genetic difference between neighbouring sites T3 and T4 may be an effect of sampling sibling groups rather than populations (Section 5.2.4).

	F_{IS} by Locus by Population					*F* Statistics by Locus		
Locus	**T2**	**T3**	**T4**	**G1**	**G4**	F_{IS}	F_{IT}	F_{ST}
EST-1	-0.055			0.291	-0.018	0.155	0.148	-0.007
EST-2								
FUM	1.000***	-0.500		-0.016	0.470*	0.764	0.745	-0.08
αGP-1								
αGP-2								
GPI		0.000		0.552**	-0.500**	-0.007	0.007	0.013
IDH-1		1.000***				1.000	1.000	0.314
IDH-2	1.000***	1.000***				1.000	1.000	0.396
MDH	0.000	-0.222		-0.201	-0.476**	-0.187	0.766	0.803
MEN			0.000	0.593***	1.000***	-0.045	0.034	0.075
PGM								
All loci	0.812***	0.265	0.000	0.204*	0.083			
All loci and all populations						0.675***	0.801***	0.387***
Resampling mean						0.660	0.793	0.377
S.E.						0.263	0.266	0.258

Table 7.2 *F* statistics for five *Wormaldia taganananana* populations. F_{IS} is calculated over all alleles at polymorphic loci in each population, and F statistics for each locus over all populations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Blank cells indicate fixed homozygous loci.

Site	T2	T3	T4	G1	G4
T2	0	0.407*	0.514*	0.339*	0.399*
T3	0.200*	0	0.132*	0.161*	0.110*
T4	0.182*	0.144*	0	0.004	0.003
G1	0.198*	0.173*	0.027	0	0.048
G4	0.221*	0.135*	0.039	0.063	0

Table 7.3 Inter-population genetic distances for *Wormaldia taganananana*. Above the diagonal: θ, an estimator of F_{ST} (Weir and Cockerham, 1984); below the diagonal: Rogers' genetic distance (1972). * $p < 0.05$.

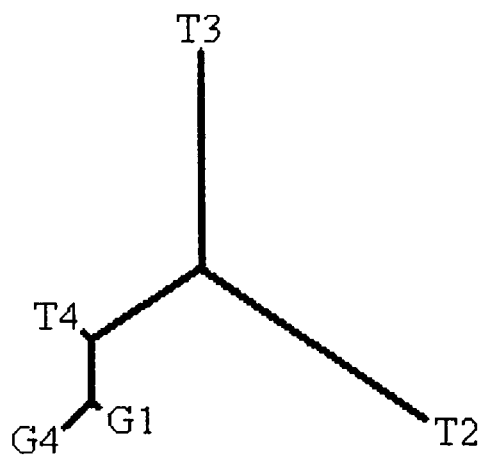


Figure 7.2 Distance Wagner network of genetic distances between populations of *Wormaldia tagananana.* Rogers' (1972) genetic distance was used in the distance Wagner procedure; dendrogram redrawn as an unrooted network.

7.3.3 Genetic distance and geographical isolation

Regressions of pair-wise F_{ST} and Rogers' genetic distance against geographic distance between sites were positive but not significant (Figure 7.3). Mantel tests on each pair of matrices confirmed that there was no significant pattern of isolation-by-distance.

7.4 Discussion

7.4.1 Genetic variability compared to more widespread species

Genetic variation in *Wormaldia tagananana* was broadly similar to those found in previous published studies of continental Trichoptera (Table 6.4). This suggests that, although an endemic species with few extant populations, *W. tagananana* has not been affected largely by genetic bottlenecks than populations of continental species (Carson and Templeton, 1984). However, genetic variation within W. *tagananana* was significantly lower than that found for *Mesophylax aspersus* (Table 6.1). Genetic variation was particularly low in the population at T4, most likely due to the small number of individuals sampled. Two site-specific alleles and one island-specific allele were found, suggesting that there is little mixing of populations and that they may have a long history of isolation. Genetic drift and other stochastic processes will have the greatest effect on low-vagility taxa (Haydon *et al.*, 1993). This is in contrast to the interpopulation dispersal of *M. aspersus* implied by the high genetic variability found within *M. aspersus* populations and the relative lack of site- and island-specific alleles.

The relative levels of genetic variation in *W. tagananana* and *M. aspersus* are consistent with a positive relationship between variation and range size, the two being linked by dispersal, affecting both the level of gene flow and the probability of new sites being colonised. In contrast, Plague and McArthur (1998), studying allozyme variation in

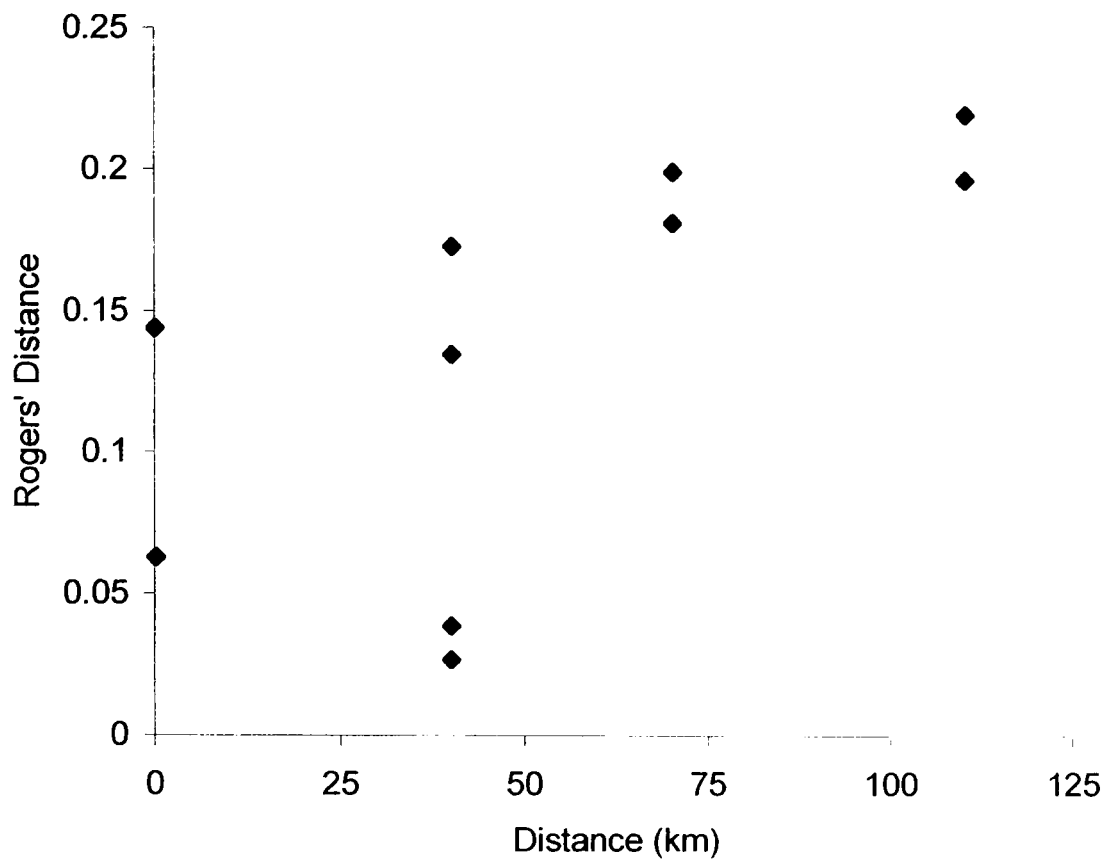


Figure 7.3 Rogers' genetic distance plotted against geographic distance between five populations of *Wormaldia tagananana.* See Figure 6.2 for an explanation of the 'paired' distance measurements.

adults of five species of *Cheumatopsyche* (Trichoptera: Hydropsychidae), found that genetic diversity was not correlated with size of geographic distribution. Their conclusion was based on the finding that *C. petiti*, with a continent-wide North American distribution, had as low genetic diversity as the local endemic *C. richardsoni*. However, only one population per species was sampled, so small sample size or a local bottleneck could have produced this result.

7.4.2 Population structure: genetic and geographic isolation

Highly significant population structure was found in *Wormaldia tagananana*, as predicted, with some evidence of greater inter-population dispersal within islands than across islands provided by the similarity between G1 and G4. Significant genetic distances were found between most pairs of populations. Together these results are evidence for very limited, or a complete lack of, interpopulation dispersal. This is in contrast to the higher degree of interpopulation dispersal of *Mesophylax aspersus* implied by the lower F_{ST} values, and the relative lack of site- and island-specific alleles of that species (Chapter 6).

F_{IS} was significantly positive overall, but varied in sign from locus to locus, as was found for *M. aspersus*. A combination of selection at some loci, counteracting an overall tendency towards homozygosity through genetic drift and inbreeding in small populations may explain these results (Section 6.4.2). F_{IS} and F_{ST} were greater in *W. tagananana* than *M. aspersus*, that is, *W. tagananana* is more highly structured. Deviation from Hardy-Weinberg equilibrium within populations (high F_{IS}) may also be due to the individuals sampled from each site being the product of a limited number of mating events (*i.e.* sampling error rather than inbreeding).

Population differentiation has been used in several studies to make inferences about the movements of individuals (*e.g.* Bohonak, 1999b), but alternative explanations should be eliminated. Firstly, population differentiation reflects the history of population bottlenecks and historical in addition to current gene flow, *i.e.* the 'relatedness' of populations (*e.g.* Boileau *et al.*, 1992; Bossart and Prowell, 1998), and is particularly important where there is no ongoing dispersal. However, the trend towards isolation-by-distance irrespective of island boundaries is evidence for limited, yet consequential, ongoing dispersal in *W. tagananana*. Secondly, natural selection may have produced the observed allozyme variation, and, as geographically close populations will tend to have similar environments, an association between genetic and geographical distances might arise through a common association with 'environmental distances' (Manly, 1986). However, there is no direct evidence for this in the present study, as selection is not the most parsimonious explanation (Varvio-Aho, 1983; Harrison and Hastings, 1996), the *W. tagananana* populations are differentiated at a number of loci, and the streams encompass a smaller range of environmental variation than encountered in other similar studies (*e.g.* Waples, 1987).

7.4.3 Genetic differentiation and dispersal

Comparative studies are required to determine the relative effects of historical contingency, natural selection and genetic drift/gene flow. As a generalisation, studies on groups of species differing in dispersal ability/vagility have demonstrated that species with greater dispersal abilities show less population differentiation than those with reduced dispersal ability (Waples, 1987; Bohonak, 1999a). The present study considered two species with differing distributions and, assuming allozyme differentiation is a reflection of past and current gene flow, attempted to relate geographical range size to species' dispersal abilities. Compared to *Mesophylax aspersus*, *Wormaldia tagananana* had low genetic

variation, highly significant population structure and a tendency towards distance-dependent dispersal. This suggests that the distribution of *W. taganananа* may be limited by the species' dispersal ability, as the long-distance dispersal required to colonise new sites on the Canary Islands is particularly infrequent. *M. aspersus* is found in almost all the streams in which *W. taganananа* occurs, and many more, allowing the possibility that the range of environmental conditions tolerated by *W. taganananа* is narrower, which would be additional to poor dispersal ability in limiting the species' distribution (Pulliam, 2000).

Chapter 8

Genetic Differentiation, Dispersal and Breeding System of the Macaronesian Endemic *Ancylus striatus* (Gastropoda: Ancylidae)

Genetic Differentiation, Dispersal and Breeding System of the Macaronesian Endemic *Ancylus striatus* (Gastropoda: Ancylidae)

SUMMARY

Allozyme electrophoresis was used to survey genetic variation and differentiation in five populations of the Canarian endemic freshwater limpet, *Ancylus striatus* (Gastropoda: Ancylidae). This species is a hermaphrodite, but the extent to which self-fertilisation occurs in natural populations is unknown. Genetic variation was moderate (mean percentage polymorphic loci (95% criterion) = 29.77%, mean unbiased estimate of heterozygosity = 0.129). It therefore does not provide strong evidence for either obligate outcrossing or selfing/parthenogenesis. Genetic variation was lower than that found for the two species of Trichoptera studied, perhaps the result of both lower gene flow, due to reliance on passive dispersal, and inbreeding. Several loci were fixed in the heterozygous state (multilocus F_{IS} = -0.666), suggesting polyploidy or chromosomal inversions, both of which are associated with parthenogenetic reproduction. Fifteen site- or island-specific alleles were found, probably resulting from very low levels of population mixing coupled with selfing/parthenogenesis. Population structure (multilocus F_{ST} = 0.364), and genetic distances between all pairs of populations, was significant. However, a significant trend of increasing genetic differentiation with increasing geographic distance was not observed. It was concluded that interpopulation dispersal is infrequent, and is distance-independent.

8.1 Introduction

One of the most abundant and widespread species within the streams of the western Canary Islands is the endemic freshwater limpet *Ancylus striatus* Quoy and Gaimard, 1834 (Gastropoda: Ancylidae). In contrast to aquatic insects such as the Trichoptera, molluscs disperse between isolated streams and islands by passive means alone, being transported accidentally through being attached to larger animals, particularly birds, and by human activity (*e.g.* Boag, 1986; Ponder *et al.*, 1994; Bilton *et al.*, in press). A study of electrophoretic variation in selected populations of this species was performed, enabling comparison of variation and differentiation with that found for two species of Trichoptera. However, the two groups may also differ in their breeding system: the study of genetic variation of *A. striatus* allowed some inferences about the species' breeding system, and its consequent effect on colonisation ability, to be made.

Populations of molluscs are often highly structured, with significant genetic differentiation between populations. This is greatest in poorly dispersing species and in isolated populations occupying island-like habitats (Ponder *et al.*, 1994; Viard *et al.*, 1996). Population structure is often hierarchical: genetic divergence between populations within drainage systems is high, indicating low levels of gene flow and differences between drainage systems are greater, reflecting an even smaller occurrence of inter-drainage gene flow. Taxa tend to remain allopatric or parapatric, with geographically restricted distributions, suggesting that dispersal can be limited even on evolutionary timescales (Chambers, 1980; Colgan and Ponder, 1994). Several studies have found a pattern of distance-dependent population differentiation in molluscs, particularly over the smallest spatial scales where active dispersal plays a part (Dillon, 1984; Goudet *et al.*, 1994; Johnson and Black, 1995). The transient nature of some freshwater habitats is expected to

make population founding events and associated bottlenecks more frequent, decreasing genetic variation within populations (Ponder *et al.*, 1994; Jarne and Städler, 1995). In fact, average heterozygosity is generally lower in terrestrial than freshwater or marine species (Brown and Richardson, 1988), possibly due to the increased costs of locomotion for small terrestrial gastropods, reducing interpopulation dispersal still further (Denny, 1980).

The dispersal and colonisation abilities of several groups of freshwater molluscs have been well documented (Brown and Richardson, 1988; Jarne and Delay, 1991; Bilton *et al.*, in press). Dispersal mechanisms include passive transport on birds (Boag, 1986; Ponder *et al.*, 1994; Dillon and Wethington, 1995), in ships' ballast or drinking water (Bilton *et al.*, in press) and with fish and plant stocks for aquaculture and the aquarium trade (Woodruff *et al.*, 1985). Within stream systems, passive downstream drift may aid dispersal (Hynes, 1970; Elliott, 1971). Active dispersal may also occur over short distances but has a high metabolic cost (Denny, 1980), and so it is usually very limited - maximum estimates are 30-150m per individual per year (Dillon, 1988; Hughes *et al.*, 1995; Johnson and Black, 1995).

Genetic variation and population differentiation are influenced by breeding system in addition to past and current gene flow and dispersal. Many freshwater molluscs are capable of self-fertilisation or some form of asexual reproduction (*e.g.* Jarne and Delay, 1991; Jarne *et al.*, 1993; Städler *et al.*, 1993, 1995; Wethington and Dillon, 1997), and a weak relationship has been found between breeding system and measures of genetic variability (Selander and Ochman, 1983; Brown and Richardson, 1988; Jarne and Städler, 1995). Selfing generally results in heterozygote deficiencies at polymorphic loci, by creating inbred lines within populations, and genetic variability is expected to be that of an outbreeding population (*e.g.* Doums *et al.*, 1996; Viard *et al.*, 1996). Heterozygosity is also

lost because of decreased effective population size, genetic hitchhiking (analogous to low recombination rates) and selection against deleterious mutations (Carvalho, 1994; Jarne, 1995; Kreitman and Akashi, 1995). However, the 'heterozygosity paradox' is often observed in partially selfing populations: heterozygosity is higher than expected from direct estimates of the selfing rate (Jarne and Städler, 1995; Hartl and Clark, 1997). This may be a result of selfing populations having lower inbreeding depression than outcrossing populations (Jarne *et al.*, 1993), in addition to occasional episodes of outcrossing (Charlesworth and Charlesworth, 1987; Städler *et al.*, 1993; Mukaratirwa *et al.*, 1996a, b).

Where the breeding system is apomictic parthenogenesis, the genotype is maintained through generations, changed only by mutation and episodes of sexual reproduction (Carvalho, 1994). Parthenogens may have heterozygous excess (Smith and Fraser, 1976; Young, 1979) or a level of heterozygosity similar to that of outcrossers (Berger and Sutherland, 1978; Livshits *et al.*, 1984). This is because heterozygosity is protected from loss through recombination and genetic drift, and because the mating system is much more compatible with chromosomal inversions and polyploidy, which both generate heterozygosity, than is sexual reproduction. Hence, parthenogenesis is often associated with polyploidy in animals (Suomaleinen *et al.*, 1976; Hebert, 1987; Livshits *et al.*, 1984; Jokela *et al.*, 1999) because it removes two major barriers to polyploidy: a sex-chromosome-based sex-determination mechanism and normal meiosis. Parthenogenetic populations tend towards genetic uniformity, at a rate dependent upon the exact nature of the parthenogenesis and the rate at which the spread of beneficial and neutral mutations is slowed. The genetic inflexibility of parthenogenetic reproduction does not necessarily lead to low levels of genetic diversity within populations and, under selective pressure, automictic parthenogenetic populations should evolve about as efficiently as sexually reproducing ones. Intrapopulation diversity may arise by point mutations, polyploidy, and

episodes of sexual reproduction including clone hybridisation (Hebert and Moran, 1980: Hebert, 1987).

The breeding system of a species has ecological as well as genetic consequences, as the ability of a species to colonise new sites is, in part, dependent upon its breeding system. Are selfers better colonisers? Non-outcrossing reproduction is commonly found in those species that are known to be good colonisers, and one or a few individuals who are selfing certainly have a better chance of founding a population than obligate outcrossers (Selander and Ochman, 1983; Jarne and Städler, 1995; Barrett, 1998). However, this is at the cost of genetic variability, which may affect the long-term fate of the population (Colgan and Ponder, 1994). Where there is bisexual and parthenogenetic reproduction in a single species, or in two related species, the different forms usually have different distributions (Suomaleinen *et al.*, 1976). The parthenogenetic form usually has the wider distribution and extends the species' range (termed geographic parthenogenesis). Therefore, in these species, island and other outlying populations are often of the parthenogenetic form. In most cases, this form is also polyploid. The diploid bisexual form may remain only in the ancestral parts of the distributional range. A typical example of this pattern of distribution is found in *Otiorrhynchus* (Coleoptera: Curculionidae) (Suomaleinen *et al.*, 1976). However, it is not clear in which cases selfing/parthenogenesis makes species pre-adapted for colonisation success and in which it is strongly selected for in the process of population establishment. It is likely that selfers *are* better colonisers, but the review by Jarne and Städler (1995) remains inconclusive.

The freshwater limpet *A. striatus* is endemic to the Canary Islands (Malmqvist *et al.*, 1995). It is closely related to the Palaearctic species *A. fluviatilis* Müller, 1774. An allozyme survey was made of five populations of *A. striatus* from La Palma, La Gomera

and Tenerife, enabling predictions about genetic variation, population structure and gene flow, and their relationship to breeding system, to be investigated. It was predicted that genetic variability would be similar to that of other freshwater molluscs that are predominantly selfing as, whilst the breeding system of *A. striatus* is not known, *A. fluviatilis* is partially selfing (Brown and Richardson, 1988; Jarne and Städler, 1995; Städler *et al.*, 1995). The ability of one or a few selfing individuals to found a population is predicted to produce strong founder and inbreeding effects. It is expected that dispersal between streams is likely to be infrequent enough to leave populations highly differentiated (*e.g.* Chambers, 1980; Colgan and Ponder, 1994; Hughes *et al.*, 1995). As passive dispersal may occur over long as well as short distances, it is hypothesised that interpopulation genetic distances will be independent of geographic distances (Ponder *et al.*, 1994). Finally, measures of genetic variability and population differentiation were compared with those found in two trichopteran species, in order to infer the extent to which passive dispersal and self-fertilisation have affected the species' genetic diversity.

8.2 Methods

8.2.1 Study species

Ancylus striatus is both widespread and abundant on Tenerife, La Palma and La Gomera (Chapter 3), as was *Mesophylax aspersus* (Trichoptera: Limnephilidae) (Chapter 6) but has differing dispersal and breeding mechanisms. It is also found on Gran Canaria (Nilsson *et al.*, 1998) and is the only representative of the Ancylidae on the Canary Islands. There has been uncertainty as to whether the study organism on the Canaries and/or Madeira is the European species *fluviatilis* or a distinct endemic (Malmqvist *et al.*, 1993, 1995; Hughes, 1995). *A. fluviatilis* is a predominantly selfing simultaneous hermaphrodite

(Jarne and Städler, 1995) and the genetic data on *A. striatus* will be tested against the hypothesis that the breeding system is the same.

A. striatus was found in 23 of 31 permanent streams surveyed on Tenerife, La Palma and La Gomera, in a range of habitats (agricultural land, *laurisilva*, pine forest), at altitudes of 200-1560m above sea level. Malmqvist *et al.* (1995) found *Ancylus* on Tenerife in streams, madicolous habitats, aqueducts, disconnected streambed pools, and springs. *A. striatus* was found at densities of up to $940m^{-2}$ (site T4, Tenerife, April 1998) and Malmqvist *et al.* (1993) calculated a mean density for permanent streams on Tenerife of $1.5m^{-2}$ (October/November 1991).

8.2.2 Localities and sampling

In April 1999, specimens of *Ancylus striatus* were collected from shallow pools in a set of five streams on three islands (La Palma, La Gomera and Tenerife), chosen to allow comparisons within and between catchments and islands (Figure 8.1). The study streams were P10, P11, T2, G1 and G4. They are a tributary and the main channel at Barranco del Rio, La Palma, Ijuana, Tenerife, and a tributary and the main channel at El Cedro, La Gomera, respectively (Section 2.2.1). In an attempt to sample from a single population, individuals were collected from 2-3 shallow pools in a 5-10m stretch of stream (sample size 22-43). Specimens were kept alive in insulated flasks of stream water then transferred to individual cryotubes within 2-3 hours, for storage at -196°C until analysis.

8.2.3 Electrophoretic analysis

Staining methods were devised for nineteen enzyme systems using cellulose acetate gel electrophoresis (protocol modified from Hebert and Beaton, 1991); loci of 15 of these systems could be scored reliably in the majority of populations (a total of 34 putative loci).

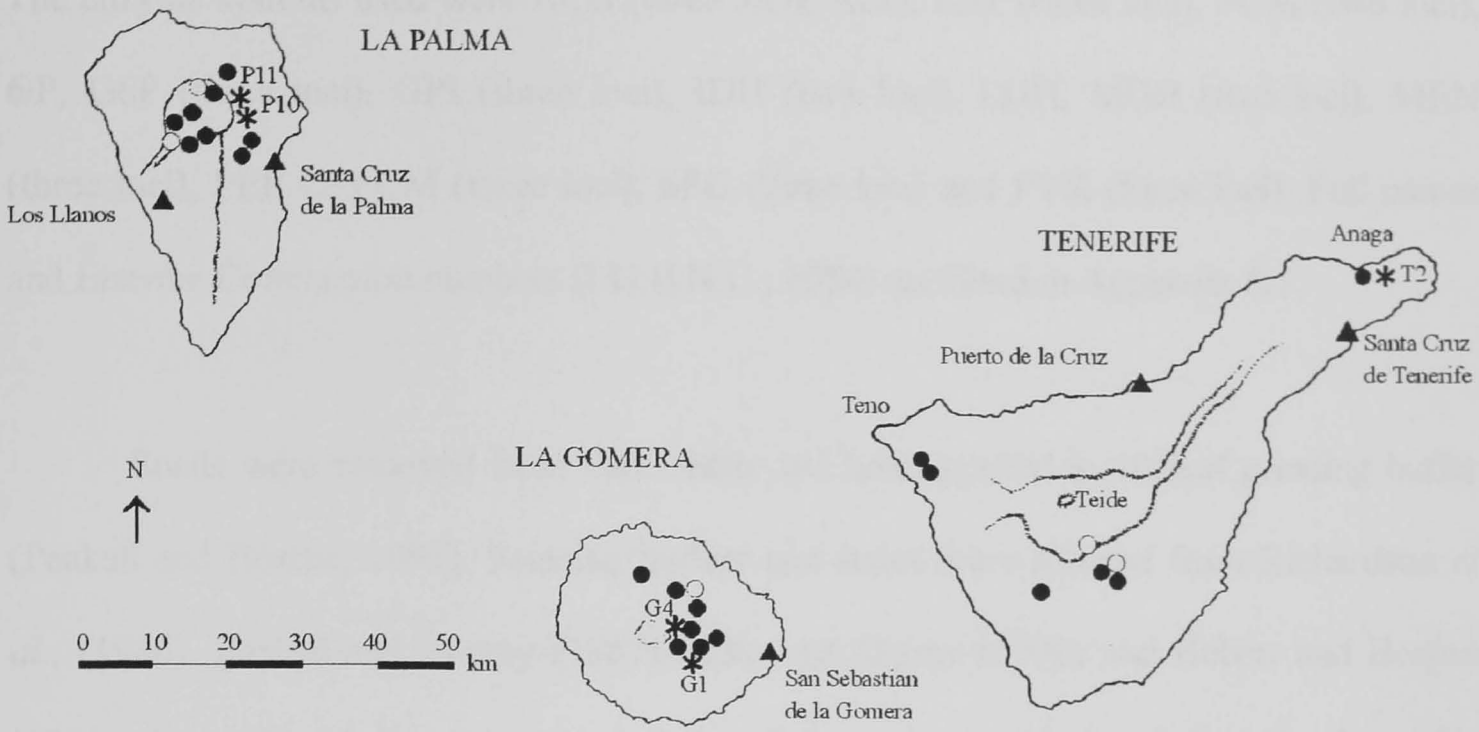


Figure 8.1 The distribution of *Ancylus striatus* in permanent streams on the western Canary Islands. ●: Species present; ○: species absent; ✳: species present and population sampled; ▲: major town or city.

The enzyme systems used were ACO (three loci), ALD, EST (three loci), FUM (two loci), GP, G6P (three loci), GPI (three loci), IDH (two loci), LDH, MDH (two loci), MEN (three loci), PEP C, PGM (three loci), 6PG (three loci) and PYK (three loci). Full names and Enzyme Commission numbers (I.U.B.N.C., 1984) are listed in Appendix 8.1.

Snails were removed from their shells and homogenised in 100µl of grinding buffer (Peakall and Beattie, 1991). Running buffers and stains were adapted from Richardson *et al.*, (1986), Easteal and Boussy (1987), Hillis and Moritz (1990) and Hebert and Beaton (1991). Appendix 6.1 lists reagents used in cellulose acetate gel electrophoresis; Appendix 6.2 lists composition of buffer solutions used; Appendix 8.1 gives the specific staining methods developed for *Ancylus striatus*, with running buffer used, run time and incubation time. Run times varied from 10-40min and incubation times from 5min-1h. Rat liver tissue (adult male Sprague-Dewley rats) was run in one lane on each gel as a positive control. Loci and alleles were labelled numerically and alphabetically respectively, in ascending order from the least to the most mobile.

8.2.4 Statistical analysis

The data were summarised as allele frequencies at each locus in each population with the BIOSYS-1 package (Swofford and Selander, 1981, 1989). As measures of genetic variability, the mean number of alleles (*MNA*) per locus, the percentage of polymorphic loci (*P*) at the 95% level and expected heterozygosity (*H*) (Neiš 1978 unbiased estimate) were calculated with BIOSYS-1. The breeding system of *Ancylus* was inferred by comparison of heterozygosity with data published for freshwater molluscs of known breeding system.

Population differentiation and structure was investigated with *F* statistics (Wright. 1951, 1969) estimated by the formulae of Weir and Cockerham (1984) with the GENETIX package (Université de Montpellier II, 1999). Whilst the assumptions underpinning *F* statistics are broken if the species is not outcrossing, the method remains a useful tool for analysis of population differentiation (*e.g.* Foltz *et al.*, 1982; Hebert and Payne, 1985; Mulvey *et al.*, 1988; Jarne and Städler, 1995; Viard *et al.*, 1997). Standard deviations of the multilocus *F* statistic estimates were obtained by jack-knifing over loci. Comparing the observed means to the outcomes generated from permutation tests estimated significance: to test F_{IS}, alleles were randomised within populations; to test F_{ST}, individual genotypes were randomly allocated to populations. Multilocus F_{ST} was calculated for each pair of sites. Pair-wise site comparisons were also performed using Nei's (1972) genetic distance. Significance of pair-wise multilocus F_{ST} was estimated by comparing the observed distances with a null distribution generated by recalculating the distance matrix after 1000 random reassignments of individuals to sites, in GENETIX. Rogers' (1972) genetic distance could not be used, and therefore distance Wagner trees could not be produced, because many of the loci (21/34) were not scored in all populations.

Multilocus F_{ST} and Nei's genetic distance for each pair of sites were plotted against geographical distance, defined as the shortest measurements on the map between sites (Figure 6.2). The relationships between the genetic and geographic distances were tested formally with Mantel tests (Mantel, 1967; Manly, 1986; Smouse *et al.*, 1986) in the GENETIX package.

8.3 Results

8.3.1 Genetic variability measures

The genetic variability showed a striking pattern, characterised by fixed homozygous and fixed heterozygous loci (Table 8.1). The frequency of occurrence of these two states characterises the five populations, in addition to the usual differences in allele frequencies within polymorphic loci. For example, the allele at PEP C is fixed across sites whilst MEN-1 is fixed in the heterozygous condition at every site. PGM-3 is fixed homozygous at T2, G1 and G4 but appears to have a fixed null (or non-staining) allele on La Palma (treated as a missing value in subsequent analyses, for consistency). MDH-1 and MEN-2 are heterozygous on La Gomera but homozygous on La Palma and Tenerife. The genotypes observed included cases of 'complex heterozygosity' (Suomaleinen *et al.*, 1976; Städler *et al.*, 1993), for example at MDH-2 at site G4, some individuals produced bands for all four alleles A-D. Subsequent analyses use allele frequencies, stated as a proportion of the number of allele records for each locus (summing to one), and so the data are treated in the same way at both 'simple' and 'complex' loci. The summary statistics that follow do not fully represent this unusual pattern of genetic variation (fixed heterozygosity is displayed in Table 8.2, F_{IS} = -1). However, the large number of loci scored reduces distortion of the summary statistics by any particular locus.

Mean *MNA* in each population (not including putative fixed null alleles) was 1.327 (range 1.2-1.524) (Table 8.1). Mean polymorphism (*P*) at non-null loci was 29.77% (range 20-40%). Mean *H* was 0.129 (range 0.085-0.161). The standard deviation of *H* is large because the data set includes rare alleles at some loci and fixed heterozygosity at others. *MNA*, *P* and *H* are lowest at T2 and highest at G1 and G4, due to the presence of additional

Locus	Allele	P10	P11	T2	G1	G4	Locus	Allele	P10	P11	T2	G1	G4
ACO-1	(N)	14	21	0	22	0	G6P-3	(N)	0	1	22	22	0
	A	0.643	0.5		1			A		0	1	1	
	B	0.357	0.5		0			B		1	0	0	
ACO-2	(N)	30	21	22	16	0	GPI-1	(N)	0	0	22	22	0
	A	1	1	1	1			A			1	1	
ACO-3	(N)	6	11	0	22	0	GPI-2	(N)	27	17	22	13	22
	A	1	1		1			A	1	1	1	1	0.864
								B	0	0	0	0	0.046
ALD	(N)	35	21	1	22	1		C	0	0	0	0	0.09
	A	0.888	0.667	1	1	1							
	B	0.114	0.333	0	0	0	GPI-3	(N)	16	18	22	22	0
								A	1	1	1	1	
EST-1	(N)	38	10	14	22	18							
	A	1	1	1	1	1	IDH-1	(N)	31	22	20	22	21
								A	0.726	1	1	1	1
EST-2	(N)	43	22	22	21	22		B	0.274	0	0	0	0
	A	0.5	0.75	1	0.738	0.864							
	B	0.5	0.25	0	0.262	0.136	IDH-2	(N)	43	22	20	22	20
								A	1	1	1	1	1
EST-3	(N)	30	21	0	10	18							
	A	1	1		0.95	1	LDH	(N)	40	18	8	7	0
	B	0	0		0.05	0		A	1	1	1	1	
FUM-1	(N)	30	3	0	17	15	MDH-1	(N)	43	21	17	21	19
	A	0	0.333		0.5	0.867		A	1	1	1	0.5	0.5
	B	1	0.667		0.5	0.133		B	0	0	0	0.5	0.5
FUM-2	(N)	38	3	0	14	21	MDH-2	(N)	43	21	21	20	20
	A	1	0.667		0.75	1		A	0.5	0.524	0.381	0.5	0.5
	B	0	0.333		0.25	0		B	0.5	0.476	0.619	0	0.075
								C	0	0	0	0.025	0.175
AGP	(N)	35	12	19	22	22		D	0	0	0	0.475	0.25
	A	0.5	1	1	1	1							
	B	0.5	0	0	0	0	MEN-1	(N)	43	21	18	7	6
								A	0.5	0.5	0.5	0.5	0.5
G6P-1	(N)	35	21	22	22	0		B	0.5	0.5	0.5	0.5	0.5
	A	0	0	0.205	0.5								
	B	1	1	0.795	0.5		MEN-2	(N)	38	9	14	21	20
								A	1	1	1	0.738	0.5
G6P-2	(N)	27	22	10	22	1		B	0	0	0	0.262	0.5
	A	0.407	0	0.15	0.091	0							
	B	0.593	1	0.85	0.909	1	MEN-3	(N)	43	22	0	0	0
								A	1	1			

Table 8.1 Allele frequencies in five *Ancylus striatus* populations. Alleles labelled A to D at each locus. (N): number of individuals for which the locus was scored; *MNA*: mean number of alleles scored per locus; *P*: percentage of polymorphic loci at 95% criterion; *H*: unbiased estimate of expected heterozygosity.

Locus	Allele	P10	P11	T2	G1	G4
PEP C	(N)	38	22	19	22	22
	A	1	1	1	1	1
PGM-1	(N)	5	0	0	21	22
	A	1	1		1	0.864
	B	0			0	0.136
PGM-2	(N)	27	14	6	0	0
	A	1	1	1		
PGM-3	(N)	0	0	12	21	22
	A			1	1	1
6PG-1	(N)	43	21	16	0	0
	A	1	1	1		
6PG-2	(N)	5	0	0	0	0
	A	1				
6PG-3	(N)	5	22	0	21	21
	A	1	1		1	1
PYK-1	(N)	13	0	10	21	11
	A	1		1	1	1
PYK-2	(N)	39	0	1	21	15
	A	1		1	1	1
PYK-3	(N)	22	22	10	0	0
	A	1	0.5	0.5		
	B	0	0.5	0.5		
MNA		1.258	1.276	1.2	1.379	1.524
S.D.		0.445	0.455	0.408	0.561	0.814
P (95%)		25.81	28.57	20.00	34.48	40.00
H		0.116	0.141	0.085	0.140	0.161
S.D. (*H*)		0.207	0.229	0.180	0.216	0.228

Table 8.1 Continued. Alleles labelled A to D at each locus. (N): number of individuals for which the locus was scored; *MNA*: mean number of alleles scored per locus; *P*: percentage of polymorphic loci at 95% criterion; *H*: unbiased estimate of expected heterozygosity.

alleles on La Gomera. In total, there were seven island-specific and eight site-specific alleles in the population samples surveyed.

8.3.2 Population structure: genetic and geographic isolation

The highly negative F_{IS} (-0.666) indicates the excess of heterozygosity in individuals, given the gene pool of the population to which they belong (Table 8.2). The heterozygote excess was highly significant in every population. When F_{IS} is calculated across the populations it is negative at almost every variable locus. Significance of individual F_{IS} could not be calculated due to the number of blank cells in the table. The negative multilocus F_{IT} (-0.056) indicates that individual genotypes have an excess of heterozygosity relative to the total gene pool. The significantly positive F_{ST} (0.364) suggests strong population structuring in *Ancylus striatus*.

Interpopulation genetic distances were generally highly significant (Table 8.3). However no significant relationship was found between geographical distance and genetic distance (Mantel test of both F_{ST} and Nei's distance against geographic distance between sites), though there was a trend of increasing genetic differentiation with increasing isolation (Figure 8.2). (F_{ST}: 71/1000 permutations give $Z \geq 463.54$ observed; Nei's D: 244/1000 permutations give $Z \geq 512.20$ observed).

	F_{IS} by Locus by Population					*F* Statistics by Locus		
Locus	**P10**	**P11**	**T2**	**G1**	**G4**	F_{IS}	F_{IT}	F_{ST}
ACO-1	-0.529	-1		fixed		-0.823	-0.214	0.334
ACO-2	fixed	fixed	fixed	fixed				
ACO-3	fixed	fixed		fixed				
ALD	-0.115	-0.481	fixed	fixed	fixed	-0.087	-0.315	0.209
EST-1	fixed	fixed	fixed	fixed	fixed			
EST-2	-1	-0.313	fixed	-0.333	-0.135	-0.831	-0.217	0.335
EST-3	fixed	fixed		0	fixed	0	0	0
FUM-1	fixed	1		-1	-0.12	-0.132	0.893	0.905
FUM-2	fixed	1		-0.3	fixed	0	0	0
AGP	-1	fixed	fixed	fixed	fixed	-1	-0.086	0.457
G6P-1	fixed	fixed	-0.235	-1		-0.240	0.056	0.239
G6P-2	-0.677	fixed	0.64	-0.077	fixed	-0.454	-0.279	0.120
G6P-3		fixed	fixed	fixed				
GPI-1			fixed	fixed				
GPI-2	fixed	fixed	fixed	fixed	0.276	0.274	0.336	0.085
GPI-3	fixed	fixed	fixed	fixed				
IDH-1	-0.364	fixed	fixed	fixed	fixed	-0.359	-0.036	0.238
IDH-2	fixed	fixed	fixed	fixed	fixed			
LDH	fixed	fixed	fixed	fixed				
MDH-1	fixed	fixed	fixed	-1	-1	-1	0.118	0.559
MDH-2	-1	-0.905	-0.6	-0.905	-0.517	-0.771	-0.499	0.153
MEN-1	-1	-1	-1	-1	-1	-1	-1	0
MEN-2	fixed	fixed	fixed	-0.333	-1	-1	0.087	0.544
MEN-3	fixed	fixed						
PEP C	fixed	fixed	fixed	fixed	fixed			
PGM-1	fixed	fixed		fixed	-0.135	-0.120	-0.077	0.038
PGM-2	fixed	fixed	fixed					
PGM-3			fixed	fixed	fixed			
6PG-1	fixed	fixed	fixed					
6PG-2	fixed							
6PG-3	fixed	fixed		fixed	fixed			
PYK-1	fixed		fixed	fixed	fixed			
PYK-2	fixed		fixed	fixed	fixed			
PYK-3	fixed	-1	-1			-1	0.231	0.615
All loci	-0.771***	-0.569***	-0.672***	-0.716***	-0.229***			
All loci and all populations						-0.666***	-0.056***	0.364***
Re-sampling mean						-0.666	-0.060	0.364
S.E.						0.177	0.195	0.136

Table 8.2 F statistics for five *Ancylus striatus* populations. F_{IS} was calculated over all alleles at polymorphic loci in each population, and F statistics for each locus over all populations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Blank cells indicate loci that were not scored (in contrast to Tables 6.2 and 7.2). 'Fixed' indicates loci that are fixed homozygotes (F_{IS} cannot be calculated); where $F_{IS} = -1$, loci are fixed heterozygotes.

	P10	P11	T2	G1	G4
P10	0	0.237*	0.243*	0.332*	0.384*
P11	0.148**	0	0.333	0.367*	0.264*
T2	0.307**	0.296**	0	0.248*	0.279*
G1	0.246**	0.303**	0.235**	0	0.090*
G4	0.413**	0.519**	0.427**	0.223**	0

Table 8.3 Interpopulation genetic distances for *Ancylus striatus*. Above the diagonal: θ an estimator of F_{ST} (Weir and Cockerham, 1984); below the diagonal: Neiš (1972) genetic distance (* $p < 0.01$, ** $p < 0.001$).

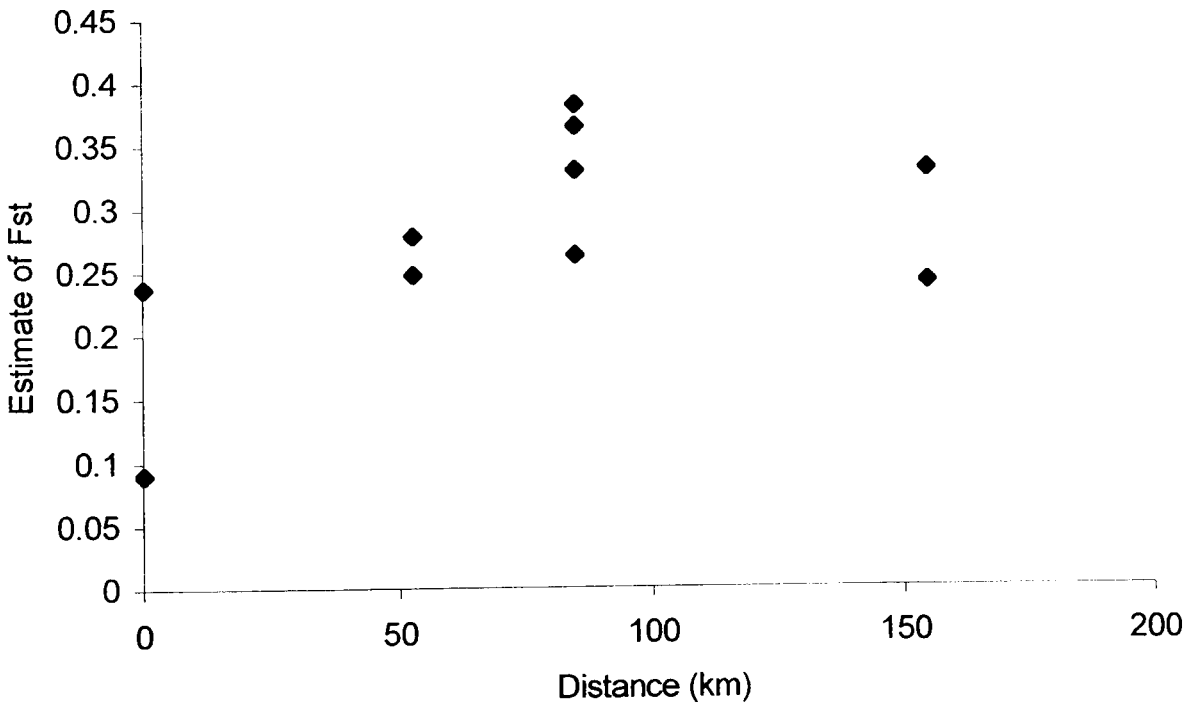


Figure 8.2 Scatterplot showing genetic and geographic distance between five populations of *Ancylus striatus*. See Figure 6.2 for explanation of measurement of genetic distances.

8.4 Discussion

8.4.1 Genetic variation

The standard genetic variability measures *MNA*, *P* and *H* varied from site to site, with T2 always having the lowest genetic diversity and the sites on La Gomera having the most. Those loci which were not fixed often differed markedly in their heterozygosity from that predicted by the observed allele frequencies, particularly where $H_{obs} = 1$, in which case $H_{exp} \approx 0.5$. The loci that were not scored may be null allele homozygotes, as non-functional alleles are known to occur in molluscs, particularly in the case of polyploid organisms (the 'gene-silencing' effect) (Jarne and Städler, 1995).

Variation in genetic diversity between populations may be explained by differing effective population sizes, differing amounts of time having elapsed since population founding or other bottlenecks, and by bottlenecks of differing severity (Hebert and Moran, 1980). The populations may also have differing probabilities of receiving migrants. Finally, G1 and G4 could be source or parent populations for T2, P10 and P11, but not vice versa, because of the additional alleles present on La Gomera. This is not consistent with the geological ages of the islands (Tenerife > La Gomera > La Palma) but populations may have gone through many cycles of local extinction and recolonisation since the first colonisation of the islands by the species.

The mean values of *MNA*, *P* and *H* are typical for freshwater molluscs, though *H* is slightly lower than would be usual for an outcrossing species (Table 8.4). The high level of 'private' alleles (Slatkin, 1985b) is indicative of genetic drift (due to low gene flow), potentially reinforced by self-fertilisation (Njiokou *et al.*, 1993).

Species[a]	Breeding System	Habitat	Scale (km)	Pops.	Loci	*N*	*MNA*	*P* %	*H* (obs.)	F_{IS}	F_{ST}	Ref.[b]
Ancylus striatus	Mixed	Streams	150	5	33	1-43	1.327	29.8	0.200	-0.666	0.364	14
Biomphalaria glabrata	Selfing	Streams		7	26			15	0-0.06			3
Biomphalaria glabrata	Selfing	Streams	1000	6	13						0.805	4
Biomphalaria pfeifferi		Streams	500	12	7						0.589	5
Biomphalaria straminea	Possibly outcrossing	Streams		4	19		1.421	26	0.056-0.097	0.044	0.098	3
Biomphalaria spp.		Streams		25	22			28.7 (4.5-63.6)	0.052 (0.002-0.126)			10
Bulinus globosus	Mixed	Streams		27	8			75			0.011	13
Bulinus globosus	Mixed	Streams		8					0.06-0.26			12
Fluvidona spp.		Streams	30								0.075-1.104	7
Fluvidona spp.		Streams	15	65	22	50-100	1.0-2.1	0-63.6	0-0.23		0.03-0.59	9
Goniobasis sp.		Streams	1000	12	14						0.554[c]	1
Lymnaea peregra		Ponds	50	4	6						0.018	6
Lymnaea peregra		Ponds	450	11				73.5 (45.4-100)	0.243 (0.159-0.458)	0.321 (0.131-0.894)	0.215 (0.055-0.338)	8
Melanoides tuberculata	Partheno-genetic	Streams		4	>6	24-56		50	0.111 (0-0.227)			2
Melanoides tuberculata	Outcrossing	Streams		5	>6	24-69		50	0.326 (0.18-0.516)			2
Physa hetero-stropha	Outcrossing	Streams	10	10	10					0.198	0.306	11

[a] Families represented: Ancylidae (*Ancylus*); Hydrobiidae (*Fluvidona, Fonscochlea, Trochidrobia*); Lymnaeidae (*Lymnaea*); Melaniidae (*Melanoides*); Physidae (*Physa*); Planorbidae (*Biomphalaria, Bulinus*); and Pleuroceridae (*Goniobasis*).

[b] References: 1: Chambers (1980); 2: Livshits *et al.* (1984); 3: Woodruff *et al.* (1985); 4: Mulvey *et al.* (1988); 5: Bandoni *et al.* (1990); 6: Jarne and Delay (1990); 7: Colgan and Ponder (1994); 8: Coutellec-Vreto *et al.* (1994); 9: Ponder *et al.* (1994); 10: Bandoni *et al.* (1995a); 11: Dillon and Wethington (1995); 12: Mukaratirwa *et al.* (1996a); 13: Mukaratirwa *et al.* (1996b); 14: the present study. Further studies are reviewed by Selander and Ochman (1983), Brown and Richardson (1988), Jarne and Delay (1991), Jarne *et al.* (1993), Jarne (1995) and Jarne and Ståler (1995).

[c] Nei's (1977) G_{ST}, approximating F_{ST}.

Table 8.4 Genetic variation and population differentiation in selected species of freshwater Mollusca. Breeding system is given where it is known independently of population genetic data. Mean and range of parameters are given, where available.

8.4.2 Genetic differentiation and population structure

F_{IS} for *Ancylus striatus* is consistently negative, indicating the extent of the excess of heterozygosity. Natural selection may maintain some of the excess heterozygosity observed (Young, 1979; Carvalho, 1994). The allozyme loci themselves might not be under strong selection but be in linkage disequilibrium with another gene that is selected. However, it is hard to invoke a selective force that could maintain heterozygosity to the exclusion of any homozygotes at some sites yet not at others, even in combination with selfing. The lack of consistent trends across sites is evidence against the selectionist explanation. F_{IT} is influenced by the strongly negative F_{IS}, but the difference between F_{IS} and F_{IT} indicates that population structuring also accounts for a large proportion of the variation in heterozygosity. The potential for different breeding systems to create and maintain excess heterozygosity is discussed below. However, the assumptions of *F* statistics are not met if the heterozygote excess is due to polyploidy or parthenogenesis (Wright, 1969; Hartl and Clark, 1997).

There is great variation between loci of *A. striatus* in terms of F_{ST} (range 0-0.905). Coutellec-Vreto *et al.* (1994) also found extensive locus-to-locus variation in F_{ST} in *Trochidrobia* (Hydrobiidae). The comparison of F_{ST} between studies can only be made very broadly, as the statistic is an average dependent upon the design of each sampling scheme, in particular the distance between populations relative to the species' dispersal ability. The level of population differentiation in *A. striatus* is high (multilocus F_{ST} = 0.364), though not as high as that found for self-fertilising *Biomphalaria* (Planorbidae) by Mulvey *et al.* (1988). *A. striatus* contrasts with the little population structuring found in recently founded populations of *B. straminea* by Woodruff *et al.* (1985) (as in the models of Latter (1973) and Nei *et al.* (1977)). Genetic differentiation of populations increases when habitat is discontinuous (Johnson and Black, 1991, 1995). *B. glabrata* was studied by

Mulvey *et al.* (1988) on Caribbean islands and the high genetic differentiation was primarily accounted for by inter-island differentiation (78%), with only 2% of the variation explained by intra-island differences. Although too few populations were sampled to analyse the variation in *A. striatus* hierarchically, F_{ST} was significant between sites on the same island, as it was between sites on different islands. Therefore, a lack of dispersal within, as well as between, islands is inferred. This is more similar to the results of Dillon and Wethington (1995), where none of the variance between populations of *Physa heterostropha* (Physidae) could be attributed to island/land mass by hierarchical analysis of *F* statistics.

8.4.3 Genetic distance, gene flow and geographic distance

Gene flow patterns are often complex, and dependent upon the interaction of current and historical factors (Colgan and Ponder, 1994; Ponder *et al.*, 1994). Estimates of gene flow from gene frequency data require that the variance in gene frequencies among populations has reached gene flow-drift equilibrium (Wright, 1943). The allele frequencies of *Ancylus striatus* are far from equilibrium and so it is not appropriate to make too much of gene flow estimates (Boileau *et al.*, 1992). In natural situations, significant gene frequency divergence need not imply low levels of gene flow (Allendorf and Phelps, 1981), and this would certainly be the case when the population dynamics are dominated by subpopulation extinction and recolonisation (Wade and McCauley, 1988).

Given the above, it is still informative to examine the estimates of population differentiation for evidence of dispersal patterns (Jarne, 1995). Gene flow in *A. striatus* is low, though interpopulation estimates of Nei's genetic distance were similar to those found for *Biomphalaria* by Bandoni *et al.* (1995a). Low gene flow means that local differentiation under selective pressures is not swamped (Dillon, 1988; Ponder *et al.*,

1994). The consequence of low gene flow is increased population differentiation, as the processes of genetic drift and local adaptation under natural selection are not counter-acted by immigration. It is not possible to differentiate between populations with low current gene flow and those with no current gene flow but shared history (Slatkin, 1985a; Dillon, 1988). Computer simulations have demonstrated that in the case where only one or a few individuals found populations, the gene frequency divergence established at the colonisation events is resistant to decay by gene flow (Boileau *et al.*, 1992). Populations of selfing organisms grow rapidly and often to large sizes so, even if interpopulation dispersal occurs, it may be relatively insignificant.

Pair-wise F_{ST} and Nei's genetic distance did not correlate significantly with geographic distance in *A. striatus*, though there was a positive trend. This lack of a significant correlation has also been observed in freshwater molluscs by Dillon and Davis (1980), Livshits *et al.* (1984), Coutellec-Vreto *et al.* (1994) and Ponder *et al.* (1994). In contrast, Dillon and Wethington (1995) and Viard *et al.* (1997) found significant isolation-by-distance regardless of island boundaries and geographic scale, respectively. Lack of isolation-by-distance is caused by stochastic colonisation and dispersal, or some other pattern determined by distance-independent factors. This may arise due to the differing accessibility of streams to birds (the most likely agents of dispersal), due to differing topography, forest cover and proximity to other resources utilised by the dispersal agents, for example (Colgan and Ponder, 1994; Ponder *et al.*, 1994).

8.4.4 Comparison with actively dispersing Trichoptera

Genetic variability in *Ancylus striatus* was very similar to that found for *Wormaldia taganananа* (Trichoptera: Philopotamidae) in terms of *MNA*, *P* and *H*, but lower than that found for *Mesophylax aspersus* (Trichoptera: Limnephilidae). *W. taganananа* had low

genetic variability compared to other species of Trichoptera, whilst that of *M. aspersus* was relatively high. Passive dispersal and selfing do not appear to have depressed genetic diversity in *A. striatus* to a level below that of a poorly dispersing outcrossing species. This may be due to the effect of polyploidy and/or parthenogenesis in protecting variability against loss through genetic drift. However, Bohonak (1999a, b) found that comparing distantly related taxa, as in this study, could bias results towards the conclusion that ongoing dispersal is unrelated to population structure. This is because, as differences in phylogenetic history, ecology and biogeography increase, confounding factors will increasingly weaken correlations between dispersal ability and population differentiation (*e.g.* Boileau *et al.*, 1992).

8.4.5 Genetic variation, breeding system and karyotype

In molluscs, levels of genetic variability are closely related to a species' breeding system. Selfing rates may been measured directly in the laboratory by parent-offspring analysis (*e.g.* Städler *et al.*, 1993, 1995; Wethington and Dillon, 1997; Jarne *et al.*, 2000), though rates often differ markedly from those in natural populations (Vrijenhoek and Graven, 1992; Städler *et al.*, 1993). In studies of natural populations, rates are often estimated indirectly from F_{IS} (*e.g.* Jarne *et al.*, 1993; Njiokou *et al.*, 1993; Coutellec-Vreto *et al.*, 1994; Viard *et al.*, 1996). Positive values of F_{IS} (*i.e.* a deficiency of heterozygotes) may also be the result of spatial or temporal variation in allele frequencies (Wahlund effect), or biparental inbreeding (Jarne, 1995; Hartl and Clark, 1997). These factors become less important as the selfing rate increases (Jarne and Städler, 1995). Jelnes (1986), studying species of *Bulinus* (Planorbidae), found reduced polymorphism in selfers, a little more in mixed-mating-system taxa and most in outcrossers. Brown and Richardson (1988) calculated the following mean observed heterozygosities for freshwater molluscs with different breeding systems: outcrossers - 0.106; facultative selfers - 0.088; selfers - not

known; parthenogens - 0.207; and overall mean - 0.131. Note the depressed heterozygosity in facultative selfers but the higher heterozygosity in parthenogens (Section 8.1).

Comparison with Table 8.4 indicates that *Ancylus striatus* has moderate levels of genetic variability, perhaps more similar to that of parthenogenetic and outcrossing species than selfers such as *Biomphalaria* (Mulvey *et al.*, 1988). Self-fertilising molluscs have been found to have greater population differentiation than outbreeding species/populations, due to the lack of homogenising interpopulation gene flow. In parthenogenetic populations of *Melanoides tuberculata*, 80% of the genetic diversity was between populations; in bisexual populations of the same species only 42% of the diversity was due to inter-population differences (Livshits *et al.*, 1984). The high F_{ST} of *A. striatus* is compatible with a selfing breeding system, but perhaps not high enough to be regarded as conclusive evidence for it. However, F_{IS} shows a marked excess of heterozygosity within individual genotypes, evidence for polyploidy and/or parthenogenesis (Hebert and Payne, 1985).

The range of breeding systems utilised by freshwater molluscs provides mechanisms for the origin and maintenance of heterozygote excess. Firstly, excess heterozygosity can be the result of polyploidy. Polyploid strains of molluscs (including *A. fluviatilis*) and other invertebrate species are known (Suomaleinen *et al.*, 1976; Jarne and Delay, 1991; Jarne and Städler, 1995) and polyploidy could explain some of the unusual allozyme banding patterns. A simple explanation for heterozygosity is, thus, that the two genomes that were united in the polyploid line were fixed for different alleles, so that the loci scored AB are actually AA+BB (Städler *et al.*, 1993; Jarne and Städler, 1995), *i.e.* allopolyploidy, as in *Bulinus truncatus* (Njiokou *et al.*, 1993a, b) and *A. fluviatilis* (Städler *et al.*, 1993). This may also explain the case at locus MDH-2: one polyploid lineage may have the genotype AA+BB, and the other AB+CD (having heterozygous parental

genomes). The AB+CD genotype could also be produced by two mutations in an autopolyploid genome. Complex heterozygosity such as this was described by Suomaleinen *et al.* (1976) who found three or four different alleles present in single individuals of tetraploid *Otiorrhynchus scaber*. Polyploidy can also explain a banding pattern at heterozygous loci where the bands are of differing intensities, for example because of an AA+AB genotype. The banding pattern of polyploid specimens describes the phenotype only, as scoring the genotype relies on interpreting band intensities, which are particularly difficult to score.

The fixed heterozygosity observed at several loci also suggests that *A. striatus* may be reproducing by apomictic parthenogenesis. A low number of genotypes can be indicative of clonal population structure, and so the apparent genetic stability of *A. striatus* is further evidence for parthenogenesis, which is known in freshwater molluscs with mixed breeding systems (Selander and Ochman, 1983; Livshits, *et al.*, 1984; Jarne and Städler, 1995). Parthenogenesis is associated with excess heterozygosity because parthenogenesis removes barriers to polyploidy, and because it avoids the loss of variation through genetic drift (Suomaleinen *et al.*, 1976; Carvalho, 1994; Jokela *et al.*, 1999). Intrapopulation diversity arises in clonal organisms through point mutations, clone hybridisation and episodes of sexual reproduction (Smith and Fraser, 1976; Hebert and Moran, 1980).

It is concluded that *A. striatus* is most likely to be tetraploid, with a flexible mating system (Foltz *et al.*, 1982; Jarne *et al.*, 1993). It is difficult to distinguish between self-fertilisation, apomictic and automictic parthenogenesis in a polyploid organism (Städler *et al.*, 1993); however, as the populations were not monogenotypic, self-fertilisation is the most likely mechanism, with occasional outcrossing generating new gene combinations (Njiokou *et al.*, 1993; Städler *et al.*, 1995). Selfing or parthenogenesis allow individuals to

found populations, avoids the genetic and energetic costs of sex, preserves local adaptation and would be strongly selected for in populations at low density and where aphallic individuals occur (Charlesworth and Charlesworth, 1987; Jarne *et al.*, 1993; Städler *et al.*, 1993). This breeding system would therefore have contributed to the wide distribution of this species on the Canary Islands (Barrett, 1998); an interesting comparison would be with the genetic variability and breeding systems of continental populations of *Ancylus fluviatilis*.

Chapter 9

Overview and Conclusions

Overview and Conclusions

9.1 Summary of results

In the largest-scale analysis, parsimony analysis of endemicity (Rosen, 1988), used to elucidate the faunal relationships between the islands, showed close faunal similarity between La Gomera and Tenerife within the Canary Islands, with Madeira quite distinct (Chapter 4). In this analysis, presence/absence data was used, that is, all species were weighted equally regardless of their commonness or rarity. The biological relationships between the islands reflected the geographical distances between islands, with faunal similarity decreasing with isolation. However, when island similarities in community composition (species abundance and constancy, rather than presence/absence) were examined, a different pattern was observed, with Tenerife being allied with La Palma (Chapter 3). The difference reflects the fact that the dominant species within islands are determined by intra-island ecological factors, such as habitat suitability and food resources, in addition to 'higher level' biogeographic patterns. La Palma had more streams than La Gomera that were physicochemically alike to those on Tenerife, accounting for the greater community similarity between streams on La Palma and Tenerife.

Trends in species richness and endemism with island biogeographical factors were investigated. Richness tended to increase with island area, altitude and geological age, and to decrease with isolation, as predicted (Chapter 3). The number of endemic species in the island species pool was greatest on Tenerife, the island with the greatest age, area and altitude, offering more opportunities for both evolution of species *in situ* and for long-term persistence of species (Chapter 4). In addition, Tenerife was the only island on which all three land use types were present, providing a greater diversity of stream habitats. However, the largest ratio of endemic species to non-endemic species occurred on the most

isolated island, Madeira, due to the low probability of non-endemic species arriving on the island. That is, whilst non-endemics are generally expected to have greater dispersal ability than endemics, on an isolated island a large proportion of species are palaeo- or neo-endemics, with few more recent (*i.e.* non-endemic) arrivals.

Significant nestedness in the stream fauna was found, with the species present at species-poor sites being subsets of those occurring at more species-rich sites (Patterson and Atmar, 1986) (Chapter 4). The nestedness is likely to have arisen due to species differing in factors, such as degree of habitat specialism or dispersal ability (Patterson, 1990), that affect their local colonisation and extinction probabilities (Lomolino, 1996). That is, the distributions of species that are habitat specialists, or poor dispersers, are nested within the distributions of more generalist and more dispersive species, which colonise a wider variety of streams. The idiosyncratic species (Table 4.4) tended to be those that occur at the more atypical sites (being species associated with colder streams, stream margins or seeps and trickles, for example). They were not necessarily rare species, for example *Ancylus striatus* and *A. fluviatilis* have both high occupancy and high abundance, but those that were excluded from the most species-rich sites.

Mean local (stream) and regional (island) species richness was significantly correlated, suggesting that individual stream communities tend to be unsaturated (*e.g.* Caswell and Cohen, 1993; Hugueny and Cornell, 2000) (Chapter 3). That is, they are limited by the size of the island species pool, which is in turn limited by opportunities for speciation through adaptive radiation and by dispersal of species onto the islands from a continental source pool. The correlation between mean local and regional richness is logically expected given the result of nestedness analysis: irrespective of the size of the island fauna, some sites contained almost all species and other contained a small

proportion of those species. It is therefore unsurprising that the mean site richness was a constant proportion of the island species pool.

In addition to species richness, macroinvertebrate community composition (species abundance and constancy, at species and family level) differed significantly between islands (Chapter 3). The physicochemistry of the Macaronesian streams was investigated, testing for significant differences between islands (Chapter 2): chemical differences between streams on the four islands were likely to be related to differences in geology, whereas differences in the physical nature of the streams were concordant with higher rainfall and lower exploitation of streams on Madeira. The community composition of the four islands was therefore predicted to reflect the physicochemistry of the streams, as well as differences in the species pools. Inter-site relationships at the two taxonomic levels were significantly correlated, suggesting that the processes producing them (habitat selection, dispersal and speciation) operate at both taxonomic levels. The latter pattern is also partly accounted for by the number of families that are represented by a single species on each archipelago.

At the mesoscale, mean species richness per stream also differed between catchment land use types (native *laurisilva* and pine forest, and deforested land) within islands (Chapter 3), though community composition (*i.e.* the most frequent and abundant species) did not. Pine forest streams supported the lowest number of species, perhaps because this is an unstable environment, and inaccessible to more recent colonists (see also Section 3.4.2). The high altitude of pine forest streams is likely to be associated with greater daily and seasonal climatic fluctuations (Chapters 1 and 2); however, the sampling scheme of the present study did not allow temporal variation in stream physicochemistry to be assessed. Physical variables differed significantly between the three catchment land use

types, reflecting the altitudinal zonation of vegetation on the islands (Section 1.2.5). Catchment land use was to a certain extent confounded with island, due to uneven replication, and so community composition of streams in different land uses may be over-ridden by the inter-island differences in community composition.

At the local scale, stream species richness was significantly correlated with calcium and magnesium ion concentrations, conductivity and pH (Chapter 3). Community composition was influenced by physicochemical variables reflecting substratum composition, flow, shade and water chemistry, with different variables being important on different islands. Stream physicochemistry affects the species present and their abundances through direct physiological responses, resource availability (*e.g.* detritus) and microhabitat availability (*e.g.* flow refugia) (Sections 2.1.1, 3.1 and 3.4.3). Generally, the variables influencing species richness and community composition differed from those that varied significantly with island and land use type (Chapter 2), suggesting that the stream invertebrate communities are influenced by the combined effects of physicochemical variation at the level of island, catchment land use and individual streams.

The abundances of a range of species commonly occurring on the Canary Islands were significantly correlated with a range of physicochemical variables, including altitude, distance from source, pH and metal ion concentrations (Chapter 3). It is these responses, of species to the local conditions, which determine their persistence and relative abundance at sites, collectively influencing the community composition.

Broad differences between endemic and non-endemic species were explored. It was inferred that endemics have greater habitat availability (or, though less likely, dispersal ability) than non-endemics, but similar niche width, as endemic species occurred, on

average, in more streams than non-endemics (*i.e.* they had higher occupancy), but were not locally more abundant (Chapter 4). The predicted positive relationship between occupancy and abundance was not found for endemic species, suggesting that the greater habitat availability does not feedback to produce greater local abundance, for example if inter-site dispersal is infrequent (Section 4.4.4).

In order to investigate specifically the role of dispersal in determining community composition (Section 1.1.3), a survey of allozyme variation (using cellulose acetate gel electrophoresis) was made for three species. These were: a Palaearctic caddisfly that is widespread on the Canary Islands, *Mesophylax aspersus* (Trichoptera: Limnephilidae) (Chapter 6); a caddisfly endemic to La Gomera and Tenerife, *Wormaldia tagananana* (Philopotamidae) (Chapter 7); and a passively dispersed Canarian endemic mollusc, *Ancylus striatus* (Gastropoda: Ancylidae) (Chapter 8). This area of the thesis was introduced with a review of the previous uses of allozyme analysis in studies of freshwater ecology and evolution (Chapter 5).

Genetic variation in the two caddisfly species was concordant with the hypothesis that the species with the more restricted distribution has its range size limited by poor dispersal ability (Section 5.2.5), as the higher level of population differentiation implied that dispersal occurs less frequently than in the other. In the widespread species, dispersal appeared to be stochastic, with inter-island dispersal events being as frequent as intra-island dispersal (Section 6.4.3). In *W. tagananana* dispersal was distance-dependent: populations that were more distant were more differentiated, regardless of island boundaries (Section 7.4.3).

Genetic analysis of *A. striatus* was dominated by the species' polyploid genome (characteristically, producing fixed heterozygosity), but suggested that self-fertilisation/parthenogenesis occur (Section 8.4.5); interpopulation dispersal was stochastic but infrequent (Section 8.4.3). The high occupancy of this species in the Canary Island streams may be due to the flexible breeding system, which gives the species good colonisation (population founding) ability, compensating for any disadvantage incurred by reliance on passive dispersal between water bodies. *A.striatus* must also be quite generalist in its habitat requirements, able to establish populations in most streams (see Section 3.4.3).

9.2 Factors affecting community composition

Ecological (*e.g.* Ricklefs and Schluter, 1993b; Begon *et al.*, 1996) and island biogeographical (*e.g.* Cody and Diamond, 1975; Grant 1998c; Whittaker, 1998) studies frequently conclude that communities are *not* randomly assembled, and the present study is no exception. Processes influencing community composition occur at a variety of scales; it has become clear that, in order to understand community assembly, an awareness of importance of scale and the use of a multi-scale approach are important (*e.g.* Frissell *et al.*, 1986; Hildrew and Giller, 1994; Poff, 1997) (Section 1.1.2). The present study examined the influence of factors on a range of scales, from the physicochemical characteristics of individual stream reaches to the faunal relationships between archipelagos, in determining Macaronesian stream invertebrate community composition (Figure 9.1).

On the largest scale, stream invertebrate assemblages reflected the biogeographic processes of island colonisation, allopatric speciation, adaptive radiation and extinction (Rosen, 1988; Clarke *et al.*, 1998; Ron, 2000). These biogeographic relationships are produced by the distribution of both endemic and non-endemic species across the islands.

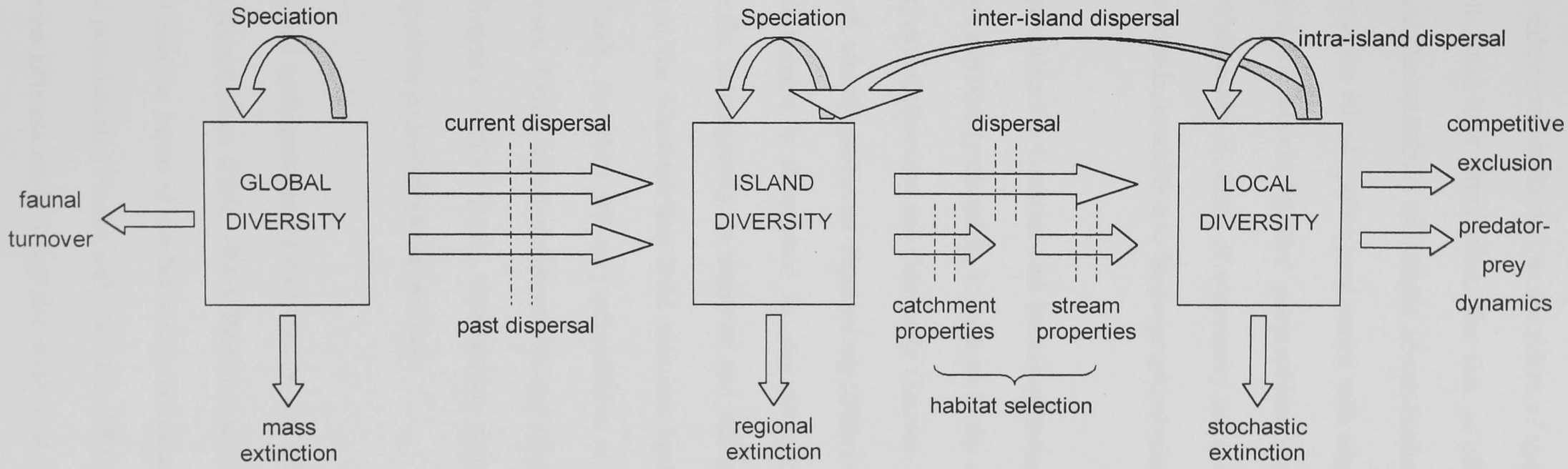


Figure 9.1 Diagrammatic representation of the forces shaping regional (island) and local (stream) species diversity. Arrows represent processes; arrows with broken lines represent 'species filters'.

Endemics have either evolved in situ, or are relicts of species with previously more widespread distributions, and their distribution, like that of other species, is limited by inter-island dispersal and the availability of suitable stream habitat. Hence, the number and proportion of endemics in the island species pool varied with island isolation, area, altitude and age. PAE arranged the islands by their geographical isolation from one another; however, inter-island relationships in terms of community composition reflected ecological similarities between the islands, in addition to biogeographical relationships.

The nestedness within the Canarian and Madeiran stream faunas provided further evidence of non-random community assembly, in contrast to the results of Malmqvist *et al.* (1997) for Canarian aquatic Coleoptera and Ostracoda (Section 1.3.4), perhaps due to the larger data set examined, and the particular dispersal capabilities of Ostracoda not found in macroinvertebrates. Nestedness is determined by colonisation dynamics, that is, it is produced by interspecific heterogeneity in dispersal and habitat selection (Figure 9.1). Significant nestedness at the Macaronesian level indicated broad similarity between the faunas of the four islands, *i.e.* they are all representative of the same regional fauna (Wright, D.H. and Reeves, 1992). Idiosyncratic species and sites within the nested pattern enhance the faunal diversity within islands, representing species with atypical habitat requirements, low competitive or high dispersal abilities.

The above regional scale processes (illustrated to the left hand side of Figure 9.1) filter through to local communities (Holt, 1993). Regional species richness constrains the maximum richness that may be found at smaller scales, but depends on the formation and extinction of individual populations (Vinson and Hawkins, 1998). The correlation between regional and local species richness implied that the number of species found in individual streams is limited by the size of island species pool.

There were significant differences in mean stream and total richness between islands and catchment land use types, and the endemic species were not evenly distributed, with diversity concentrated on Tenerife, and in *laurisilva* streams. Streams in the three land use types differed more in terms of species presence/absence than in community composition –the dominant species tended to be constant. These results again illustrate the combined effects of both biogeographical and ecological factors in producing the observed community patterns.

At the smallest scale examined (illustrated towards the right hand side of Figure 9.1), the stream reach, physicochemistry played an important part in determining stream species richness and community composition (Section 3.3.3), through species' habitat selection. Stream physicochemistry varied significantly between islands and, to a lesser extent, land use types, contributing to the community differences observed. However, the variables that showed significant relationships with the fauna were generally not those in which islands differed.

9.3 The role of species' characteristics

Characteristics of individual species might also be predicted to influence the composition of communities, for example through biotic interactions (Usseglio-Polatera *et al.*, 2000). Species differ in their environmental requirements and tolerances ('habitat selection': Figure 9.1), and biotic interactions within sites, such as facilitation, competition and predation. These differences account for the varied correlations between species local abundance and stream physicochemistry, and the relationships of community composition and species richness with physicochemistry. Departure from nestedness (Section 4.1.3) indicated species with particular characteristics, such as poor competitive ability, or

adaptation to more species-poor habitats, which exclude than from the species-rich sites. In addition, the strength of competitive interactions within an assemblage affects it invasibility. For example, it may be the case that strong competitive interactions within the *laurisilva* stream communities exclude arriving non-endemic species.

The nature of the island study system introduces two other relevant factors, endemism and dispersal/colonisation ability. Differences in occupancy and the distribution of species richness between endemics and non-endemics were likely to reflect differing habitat availability and/or dispersal ability rather than niche width or geographical range structure (Section 4.4.4). This was because greater habitat availability was inferred for the set of species with higher occupancy; endemics and non-endemics did not appear to differ in niche width, as mean local abundances were not significantly different. Regional occupancy and local abundance also appeared to be decoupled for the set of endemic species, for example, if increased occupancy is the product of a long evolutionary history on the islands, rather than resulting, through inter-site dispersal, from high local abundance (Section 4.1.5).

The dispersal ability of individual species also plays a role in determining community composition. Population genetic differentiation was used in an attempt to relate gene flow and distributional range (Chapter 7). The hypothesis tested was that the species with a larger range size (*Mesophylax aspersus*) would show more inter-population dispersal than that with a restricted distribution (*Wormaldia tagananana*). This predication had the underlying assumption that dispersal (on an evolutionary scale) that leads to the colonisation of new sites is the same process (on an ecological scale) that leads to population mixing (Patterson, 1990). The distributions of poorly dispersing taxa are likely to be dispersal limited (Pulliam, 2000); this was inferred for the endemic caddisfly

Wormaldia taganananana. As predicted, greater genetic variation, and less population differentiation, was found in the more widespread species *Mesophylax aspersus* than in *W. taganananana*. It was concluded that dispersal at the inter-island scale was likely to be just as highly stochastic as it is at smaller scales (Jeffries, 1989), and that dispersal is a factor in determining distributional range size. However, for many Macaronesian freshwater taxa dispersal may be frequent enough to prevent allopatric speciation, as there are fewer single-island endemics in the freshwater fauna than in the terrestrial fauna of these islands (*e.g.* Machado, 1992; Juan *et al.*, 2000) (Chapter 1).

Finally, the population genetic structure of a mollusc, *Ancylus striatus*, was investigated. This species relies upon passive dispersal by vectors such as birds (Boag, 1986, Bilton *et al.*, in press), as its means of reaching new stream sites. However, the genetic data strongly suggest that *A. striatus* has a mixed breeding system (*i.e.* it is not an obligate outcrosser) (Suomaleinen *et al.*, 1976; Städler *et al.*, 1993, 1995), which allows for effective colonisation, as a single individual may potentially found a new population (Chapter 8). Breeding system therefore also has an influence on community composition, with respect to certain taxa, due to its potential effect on colonisation ability.

9.4 Conclusion

The stream communities of Macaronesia are a product of processes acting over a wide range of temporal and spatial scales, from the evolution of endemics to the microhabitat characteristics encountered by individual species in the streams. Feedback between species pools at different scales influences richness at each scale (Vinson and Hawkins, 1998) (Figure 9.1), though species pools are not necessarily in equilibrium between immigration, species evolution and extinction. Many species in the fauna have

restricted or disjunctive distributions, and stream community composition is heterogeneous, varying between archipelagos, islands, catchment land use types and streams of different natures. Communities, and especially the endemic species within them, are products of the isolated island environment.

The scarcity of permanent streams on the islands, and the threats to them and pressures upon water resources, makes the species continued existence precarious. Various threats to the Macaronesian stream fauna are listed in Section 1.4; the pertinence of ecological studies on the fauna was also mentioned. The results of the present study have several implications for the conservation of Macaronesian stream invertebrates.

The abundance of individual species responds to specific physicochemical parameters (Section 3.3.3), thus species are sensitive to changes in the stream physicochemical environment (Section 2.4). The high proportion of endemics (*circa* 50%) in the Macaronesian stream macroinvertebrate fauna is especially noteworthy. These species are vulnerable to extinction because of their inherently small range sizes, and they are likely to be poorer dispersers and colonisers, and more specialised in their habitat requirements than non-endemics (Section 4.1.5). Analyses of stream occupancy and mean abundance per stream (Chapter 4) also drew attention to species vulnerable to extinction through having particularly limited range sizes (*e.g. Chaetogammarus chaetocerus* (Amphipoda: Gammaridae) and *Lepidostoma tenerifensis* (Trichoptera: Sericostomatidae)), or low abundance where they occur (*e.g. Limnebius gracilipes* (Coleoptera: Hydrophilidae).

The assemblages on different islands and in different land use types were distinct so, from a biodiversity perspective, the streams are not equivalent to one another. In

particular, *laurisilva* streams were rich in species and contained many not found in other habitats (Sections 3.3.2 and 4.3.3). Communities varied in their numbers of non-endemic species, and it appears that *laurisilva* stream communities are more resistant to the establishment of new, introduced species than others are, though this may also be a result of their geographical isolation. With respect to the islands, Tenerife was most species rich, and had more endemic species than the other islands (Sections 3.3.1 and 4.3.3). The nestedness of stream faunas, both within and across archipelagos, implies that the vast majority of species can be protected by conserving the most species-rich sites. However, there was a significant number of idiosyncratic species (Section 4.3.2), which might be missed altogether.

The genetic studies performed showed that populations were isolated, with several conservation implications. Firstly, low levels of inter-population dispersal suggest that colonisation events would be infrequent, should populations disappear. The prospects are better for species with distance-independent dispersal than for those with distance-dependent dispersal, where populations may become completely isolated. Secondly, the genetic diversity, important for the log-term survival of a lineage, within any one population is much lower than in the species as a whole, particularly in a species such as *Ancylus striatus* with a partially-selfing breeding system and no active long-distance dispersal. Species with a number of populations in close enough proximity for occasional interpopulation dispersal stand a much better chance of long term survival than do those found only in isolated populations.

The Macaronesian islands have experienced extensive environmental disturbance in the past, both climate change and volcanic activity. Indeed, this may have promoted the diversification of some groups of organisms (Juan *et al.*, 2000). Current anthropogenic

impacts on the stream environment may be equally serious though not as dramatic. Island biotas have been noted to be particularly vulnerable, and the freshwater fauna of the Canary Islands shares characteristics of endemicity, habitat specialisation and susceptibility to habitat degradation with other island biotas (Cody and Diamond, 1975; Quammen, 1996; Grant, 1998c; Whittaker, 1998; Brown and Lomolino, 2000a) (Section 1.1).

Appendices

Appendix 2.1 Water chemistry data for 42 Macaronesian streams. Concentrations of Cu, Zn, Al, Fe and PO_4 in mg l^{-1}; hardness in mg $CaCO_3$ l^{-1}; conductivity in $\mu S\ cm^{-1}$. pH was not measured for Madeiran streams.

Site	Cu	Zn	Al	Fe	PO_4	Hardness	Cond.	pH
P1	0.008	0.037	0.001	0.150	0.070	26.419	174	7.40
P2	0.001	0.009	0.001	0.128	0.060	29.114	197	7.40
P3	0.001	0.019	0.001	0.139	0.100	27.034	200	7.50
P4	0.004	0.009	0.001	0.097	0.120	30.654	87	8.24
P5	0.005	0.006	0.001	0.347	0.150	37.727	144	7.64
P6	0.001	0.009	0.001	0.131	0.150	28.928	417	7.57
P7	0.001	0.008	0.001	0.113	0.110	43.184	393	7.99
P8	0.003	0.016	0.001	0.094	0.095	138.662	141	8.67
P9	0.002	0.004	0.247	0.078	0.105	46.224	195	8.09
P10	0.005	0.007	0.121	0.267	0.175	37.607	121	7.50
P11	0.003	0.005	0.039	0.062	0.095	23.831	124	7.80
P12	0.003	0.008	0.001	0.068	0.105	12.535	245	8.40
G1	0.025	0.014	0.354	0.126	0.085	23.614	229	6.75
G2	0.009	0.007	0.560	0.142	0.060	33.096	201	6.74
G3	0.005	0.006	0.771	0.094	0.075	34.959	208	6.34
G4	0.001	0.009	1.483	0.123	0.093	43.393	208	6.30
G5	0.002	0.022	2.054	0.188	0.100	47.738	220	6.39
G6	0.001	0.006	1.899	0.177	0.080	44.510	218	6.56
G7	0.001	0.017	1.401	2.500	0.240	36.385	261	6.68
G8	0.001	0.007	1.152	0.148	0.050	34.659	937	6.80
G9	0.002	0.008	1.290	0.146	0.050	29.360	289	6.71
G10	0.005	0.007	0.477	0.297	0.020	41.600	222	6.72
T1	0.001	0.015	0.001	0.093	0.060	30.426	650	6.79
T2	0.001	0.007	0.531	0.297	0.095	55.365	353	6.86
T3	0.004	0.019	0.483	0.087	0.080	20.431	716	6.86
T4	0.001	0.014	0.642	0.062	0.068	23.492	631	6.60
T5	0.003	0.015	0.001	0.081	0.380	89.602	389	6.70
T6	0.001	0.013	0.151	0.310	0.170	95.517	378	6.66
T7	0.002	0.012	0.001	0.068	0.080	28.021	275	6.79
T8	0.002	0.017	0.001	0.066	0.095	44.322	297	6.61
T9	0.010	0.016	0.001	0.406	0.145	15.198	64	6.79
M1	0.001	0.006	1.369	0.037	0.098	15.380	78	
M2	0.005	0.007	1.543	0.066	0.060	42.416	50	
M3	0.001	0.020	1.905	0.043	0.060	68.238	92	
M4	0.014	0.015	1.521	0.100	0.095	37.159	121	
M5	0.002	0.006	0.843	0.136	0.080	144.165	132	
M6	0.003	0.007	0.581	0.067	0.110	105.047	155	
M7	0.003	0.010	0.543	0.056	0.080	68.047	140	
M8	0.005	0.011	0.598	0.029	0.080	34.350	143	
M9	0.003	0.008	0.303	0.094	0.085	25.118	133	
M10	0.001	0.003	0.001	0.040	0.090	33.719	132	
M11	0.001	0.005	0.001	0.085	0.080	5.980	117	

Appendix 2.2 Physical characteristics of 42 Macaronesian streams. Shade, gradient and flow: scale from 1 to 3; cover of substratum types and organic matter: scale from 0 to 5.

Site	Altitude (m a.s.l.)	Source (km)	Temp. (°C)	Shade	Width (cm)	Depth (cm)	Gradient	Flow	Bedrock	Boulders	Cobbles	Rocks	Gravel	CPOM	FPOM
P1	640	1.3	12.0	3	50	17	1	1	2	0	2	2	5	5	4
P2	600	2.6	12.8	3	50	17	1	1	2	0	0	2	4	5	2
P3	800	4.6	13.4	2	100	40	1	1	3	3	0	3	0	2	2
P4	1820	0	11.0	2	70	10	1	3	5	0	0	0	2	0	0
P5	1500	2	10.0	2	100	20	1	1	3	4	2	0	2	0	0
P6	840	4	14.7	2	110	20	1	3	1	2	3	3	3	1	1
P7	840	4	15.3	1	650	30	1	3	1	5	3	4	1	0	0
P8	900	0.1	15.4	1	50	15	3	1	5	4	2	2	1	0	0
P9	940	0.6	15.5	1	100	10	3	2	3	3	2	2	1	0	0
P10	920	1.8	12.9	3	40	10	2	1	4	4	0	0	3	1	1
P11	900	2.8	13.7	3	45	10	1	1	1	3	0	3	1	1	1
P12	920	0.4	15.0	1	150	40	2	2	0	5	1	2	3	1	1
G1	980	0.6	11.1	3	100	20	1	2	0	2	2	3	5	5	1
G2	990	0	11.2	3	40	15	1	1	0	4	0	0	4	4	4
G3	980	1.1	11.6	3	70	15	2	1	0	3	4	4	1	4	4
G4	990	2	11.8	3	100	20	2	2	4	0	0	3	3	3	3
G5	940	2.5	11.2	2	200	20	1	3	0	3	3	2	4	3	0
G6	1020	0.4	11.7	2	30	10	3	1	4	0	0	0	4	4	4
G7	950	0.5	11.9	2	25	10	1	1	1	0	0	5	2	4	4
G8	520	3.1	14.4	1	70	10	1	1	0	1	2	1	5	4	2
G9	830	3.6	12.5	2	100	15	1	1	0	1	5	3	2	3	2
G10	940	3.8	12.0	1	130	25	1	3	0	0	1	0	5	3	5
T1	200	3.5	16.0	1	60	30	1	2	0	2	2	2	0	4	5
T2	720	0.1	12.8	2	50	15	1	1	0	0	5	5	2	3	3
T3	360	1.8	16.5	1	100	10	2	2	5	0	0	1	2	4	0
T4	350	2.2	17.3	1	100	20	3	2	5	2	2	2	2	3	1
T5	500	5.0	13.7	2	300	20	2	3	3	4	2	2	4	2	1
T6	1520	2.8	18.5	2	40	5	1	1	0	2	1	1	5	3	5
T7	1560	3.1	14.7	1	200	30	2	3	0	5	2	1	4	1	1
T8	1400	4.4	12.5	1	150	25	1	2	0	5	3	3	4	1	1
T9	2150	0	8.7	1	30	5	3	1	5	1	0	0	5	4	3
M1	1000	5	11.0	1	400	20	1	3	2	5	3	2	1	4	0
M2	850	0.5	13.6	2	60	7	1	2	5	2	4	2	1	0	1
M3	610	1.9	13.5	3	150	15	1	2	0	5	3	3	1	4	1
M4	750	2.4	15.5	2	160	10	2	2	0	2	5	4	2	4	1
M5	400	0.9	15.2	1	60	7	3	1	0	5	4	3	2	3	1
M6	380	0.3	16.4	1	60	5	2	1	0	1	2	4	3	1	1
M7	390	1.3	15.7	2	150	20	2	3	2	5	3	3	0	1	0
M8	390	2.9	15.8	1	200	25	1	2	0	5	3	3	2	0	0
M9	150	0.75	15.5	3	200	10	3	3	0	3	5	2	1	3	0
M10	130	1.9	16.0	1	100	15	2	1	0	4	4	3	3	4	1
M11	130	1.5	15.9	2	150	7	3	2	0	3	5	3	3	0	1

Appendix 3.1 Species presence/absence records for 42 Macaronesian streams. Including: number of site records per species per island; number of species per taxonomic group (*e.g.* order) per site and island; mean number of species per group per site averaged for each island; and total number of species per site and per island.

Notes to Appendix 3.1

The groups Amphipoda, Coleoptera, Ephemeroptera, Hemiptera, Mollusca, Odonata and Trichoptera were identified to species level with the following exceptions.

Coleoptera: Larvae were identified to genus and then assigned to the species that was most abundant at the site.

Ephemeroptera: *Baetis pseudorhodani* and *B. nigrescens* could not be distinguished on the Canary Islands, and are listed as *Baetis pseudo./nigrescens*, tabulated separately from *B. pseudorhodani* on Madeira; *Cloeon* were identified to genus as there are three undescribed species on the Canary Islands, as well as *C. dipterum* - it is thought that only one species of *Cloeon* was found.

Hemiptera: An early-instar nymph of Corixidae could not be identified further.

Odonata: Early-instar nymphs could not be identified further and possibly included several species; female nymphs of *Anax* were assigned to *A. imperator*.

Trichoptera: *Hydropsyche* on the Canary Islands were not identified to species level due to confusion in the literature as to the species name (probably *H. maroccana)* — it is thought that only one species was found; *Hydroptila, Orthotrichia, Stactobia, Synagapetus, Oxyethira* on the Canary Islands and *Tinodes* on Madeira could not be identified further and possibly included several species; *Oecetis* are thought to be one species, not yet described; *Oxyethira* is represented by one species (*O. spinosella*) on Madeira, listed separately though it also occurs on the Canaries.

Taxa from genera that are monospecific on the archipelago in question were generally identified to genus level and species assigned without further confirmation.

Site	*Agabus biguttatus*	*Agabus maderensis*	*Agabus nebulosus*	*Agabus wollastoni*	*Anacaena haemorrhoa*	*Bidessus minutissimus*	*Chaetarthria similis*	*Coelostoma hispanicum*	*Cyphon gracilicornis*	*Dryops gracilis*	*Dryops luridus*	*Enochrus politus*	*Graptodytes delectus*	*Gyrinus dejeani*	*Gyrinus urinator*	*Haliplus lineaticollis*	*Helochares lividus*	*Hydraena serricollis*	*Hydrochus quadricollis*	*Hydroporus discretus*	*Hydroporus lucasi*
P1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
P2	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
P3	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
P4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P6	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
P7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P8	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
P9	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
P10	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
P11	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
P12	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
La Palma	**7**	**0**	**3**	**0**	**1**	**0**	**3**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**2**	**0**
G1	1	0	0	0	1	0	1	0	1	1	0	0	0	1	0	0	0	1	0	1	0
G2	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0
G3	1	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	1	0	1	0
G4	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0
G5	1	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	1	0	1	0
G6	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
G7	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0
G8	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	0	1	0
G9	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0
G10	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0
La Gomera	**10**	**0**	**0**	**0**	**8**	**0**	**1**	**0**	**4**	**10**	**0**	**0**	**0**	**6**	**5**	**1**	**0**	**10**	**0**	**8**	**0**
T1	1	0	0	0	1	0	1	1	0	1	0	1	0	1	1	1	1	0	1	0	0
T2	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0
T3	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	1	0
T4	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
T5	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0
T6	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
T7	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0
T8	1	0	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0
T9	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Tenerife	**6**	**0**	**3**	**0**	**2**	**1**	**3**	**3**	**0**	**9**	**0**	**2**	**1**	**2**	**5**	**3**	**2**	**1**	**2**	**2**	**1**
M1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
M8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M10	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
M11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Madeira	**0**	**1**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Site	*Laccobius canariensis*	*Laccobius gracilis*	*Laccophilus hyalinus*	*Limnebius gracilipes*	*Limnebius similis*	*Limnebius grandicollis*	*Meladema coriacea*	*Meladema imbricata*	*Meladema lanio*	*Nebrioporus canariensis*	*Nebrioporus dubius*	*Ochthebius quadrifoveolatus*	*Ochthebius rugulosus*	**Coleoptera spp. richness**	**Total island spp. richness**	*Chaetogamm. chaetocerus*	**Amphipoda spp. richness**	**Total island spp. richness**	*Baetis canariensis*	*Baetis pseudorhodani*
P1	0	0	0	0	0	0	0	0	0	0	0	0	0	**2**		0	**0**		0	0
P2	0	0	0	0	0	0	0	0	0	0	0	0	0	**3**		0	**0**		0	0
P3	0	0	0	0	0	0	0	0	0	1	0	0	0	**4**		0	**0**		1	0
P4	0	0	0	0	0	0	0	0	0	1	0	0	0	**2**		0	**0**		1	0
P5	0	0	0	0	0	0	0	0	0	1	0	0	0	**3**		0	**0**		0	0
P6	1	0	0	1	0	0	0	0	0	1	0	1	0	**7**		0	**0**		1	0
P7	1	0	0	1	0	0	0	0	0	1	0	1	0	**5**		0	**0**		1	0
P8	1	1	0	1	0	0	0	0	0	1	0	1	0	**9**		0	**0**		1	0
P9	1	0	0	1	0	0	0	0	0	1	0	1	0	**8**		0	**0**		1	0
P10	1	0	0	1	0	0	0	0	0	1	0	0	0	**6**		0	**0**		1	0
P11	0	0	0	0	0	0	0	0	0	1	0	0	0	**3**		0	**0**		1	0
P12	1	0	0	1	0	0	0	0	0	1	0	0	0	**6**		0	**0**		1	0
La Palma	**6**	**1**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**4**	**0**	**4.83**	**12**	**0**	**0**	**0**	**9**	**0**
G1	0	0	0	1	0	0	0	1	0	0	0	0	0	**10**		1	**1**		1	0
G2	0	0	0	1	0	0	0	0	0	1	0	0	0	**9**		1	**1**		1	0
G3	1	0	0	1	0	0	0	0	0	1	0	0	0	**11**		0	**0**		1	0
G4	1	0	0	0	1	0	0	1	0	1	0	0	0	**9**		0	**0**		1	0
G5	0	0	0	1	0	0	0	0	0	1	0	0	0	**9**		0	**0**		1	0
G6	0	0	0	0	0	0	0	0	0	1	0	0	0	**5**		0	**0**		0	0
G7	0	0	0	0	0	0	0	0	0	0	0	0	0	**6**		0	**0**		0	0
G8	1	0	1	0	0	0	0	0	0	1	0	0	0	**10**		0	**0**		1	0
G9	1	0	0	1	0	0	0	0	0	1	0	0	0	**9**		0	**0**		1	0
G10	0	0	0	1	0	0	0	0	0	0	0	0	0	**6**		0	**0**		0	0
La Gomera	**4**	**0**	**1**	**6**	**1**	**0**	**0**	**2**	**0**	**7**	**0**	**0**	**0**	**8.4**	**15**	**2**	**0.2**	**1**	**7**	**0**
T1	1	0	1	1	0	0	0	0	0	1	0	1	0	**16**		0	**0**		1	0
T2	0	0	0	1	0	0	0	0	0	1	0	0	0	**8**		0	**0**		1	0
T3	1	0	0	1	0	0	1	0	0	1	0	0	0	**10**		0	**0**		1	0
T4	1	0	0	1	0	0	0	0	0	1	0	1	0	**7**		0	**0**		1	0
T5	1	0	1	1	0	0	0	0	0	1	0	1	1	**12**		0	**0**		1	0
T6	1	0	0	0	0	0	0	0	0	1	0	1	1	**6**		0	**0**		1	0
T7	1	0	0	1	0	0	1	1	0	1	0	1	0	**11**		0	**0**		1	0
T8	1	0	0	1	0	0	1	0	0	1	0	1	1	**11**		0	**0**		1	0
T9	1	0	0	0	0	0	0	0	0	1	0	1	1	**8**		0	**0**		1	0
Tenerife	**8**	**0**	**2**	**7**	**0**	**0**	**3**	**1**	**0**	**9**	**0**	**7**	**4**	**9.89**	**25**	**0**	**0**	**0**	**9**	**0**
M1	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**		0	**0**		0	0
M2	0	0	0	0	0	1	0	0	1	0	1	0	0	**5**		0	**0**		0	1
M3	0	0	0	0	0	0	0	0	1	0	0	0	0	**2**		0	**0**		0	1
M4	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**		0	**0**		0	1
M5	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**		0	**0**		0	0
M6	0	0	0	0	0	0	0	0	1	0	0	0	0	**1**		0	**0**		0	1
M7	0	0	0	0	0	0	0	0	1	0	1	0	0	**3**		0	**0**		0	1
M8	0	0	0	0	0	0	0	0	1	0	1	0	0	**2**		0	**0**		0	1
M9	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**		0	**0**		0	0
M10	0	0	0	0	0	0	0	0	0	0	1	0	0	**2**		0	**0**		0	1
M11	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**		0	**0**		0	1
Madeira	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**5**	**0**	**4**	**0**	**0**	**1.45**	**6**	**0**	**0**	**0**	**0**	**8**

Site	*Baetis pseudo./nigr.*	*Baetis rhodani*	*Caenis luctuosa*	*Cloeon* sp.	**Ephemeroptera spp. richnes**	**Total island spp. richness**	*Corixidae* sp. nymph	*Gerris thoracicus*	*Hebrus pusillus*	*Hydrometra stagnorum*	*Microvelia gracillima*	*Notonecta canariensis*	*Velia lindbergi*	*Velia maderensis*	**Hemiptera species richness**	**Total island spp. richness**	*Ancylus fluviatilis*	*Ancylus striatus*	*Gyraulus parvus*	*Lymnaea truncatula*
P1	0	0	0	0	**0**		0	0	0	0	0	0	0	0	**0**		0	1	0	0
P2	0	0	0	0	**0**		0	0	0	0	0	0	1	0	**1**		0	1	0	0
P3	0	0	0	0	**1**		0	0	0	1	0	0	1	0	**2**		0	1	0	0
P4	1	0	0	0	**2**		0	0	0	0	0	0	0	0	**0**		0	0	0	0
P5	0	0	0	0	**0**		0	0	0	0	0	0	1	0	**1**		0	1	0	0
P6	1	0	0	0	**2**		0	0	0	1	0	0	0	0	**1**		0	1	0	0
P7	1	0	0	0	**2**		0	0	0	1	0	0	0	0	**1**		0	1	0	0
P8	0	0	0	0	**1**		0	0	0	0	0	0	0	0	**0**		0	0	0	0
P9	0	0	0	0	**1**		0	0	0	0	0	0	0	0	**0**		0	1	0	0
P10	1	0	0	1	**3**		0	0	0	0	0	0	1	0	**1**		0	0	0	0
P11	0	0	0	0	**1**		0	0	0	0	1	0	1	0	**2**		0	1	0	0
P12	1	0	0	0	**2**		0	0	0	0	0	0	1	0	**1**		0	1	0	0
La Palma	**5**	**0**	**0**	**1**	**1.25**	**3**	**0**	**0**	**0**	**3**	**1**	**0**	**6**	**0**	**0.83**	**3**	**0**	**9**	**0**	**0**
G1	1	0	0	0	**2**		0	0	0	0	1	0	1	0	**2**		0	1	0	0
G2	1	0	0	0	**2**		0	0	0	0	1	0	1	0	**2**		0	1	0	0
G3	1	0	0	0	**2**		0	0	0	0	1	0	1	0	**2**		0	1	0	0
G4	1	0	0	0	**2**		0	0	0	0	0	0	1	0	**1**		0	1	0	0
G5	1	0	0	0	**2**		0	0	0	1	1	0	1	0	**3**		0	1	0	0
G6	0	0	0	0	**0**		0	0	0	0	0	0	1	0	**1**		0	0	0	0
G7	0	0	0	0	**0**		0	0	0	0	1	0	1	0	**2**		0	1	0	0
G8	0	0	0	1	**2**		0	0	0	1	0	0	1	0	**2**		0	1	0	1
G9	0	0	0	0	**1**		0	0	0	1	1	0	1	0	**3**		0	1	0	1
G10	0	0	0	0	**0**		0	0	1	1	1	0	1	0	**4**		0	1	0	1
La Gomera	**5**	**0**	**0**	**1**	**1.30**	**3**	**0**	**0**	**1**	**4**	**7**	**0**	**10**	**0**	**2.20**	**4**	**0**	**9**	**0**	**3**
T1	1	0	1	1	**4**		1	1	0	1	0	0	1	0	**4**		0	1	0	1
T2	0	0	0	0	**1**		0	0	0	0	1	0	1	0	**2**		0	1	0	0
T3	1	0	0	1	**3**		0	0	0	1	1	1	1	0	**4**		0	1	0	1
T4	1	0	1	1	**4**		0	0	0	1	0	0	0	0	**1**		0	1	0	1
T5	1	0	1	1	**4**		0	0	0	1	0	1	0	0	**2**		0	1	0	1
T6	0	0	0	0	**1**		0	0	0	1	0	0	0	0	**1**		0	0	0	0
T7	1	0	1	1	**4**		0	0	0	1	0	1	0	0	**2**		0	1	0	0
T8	1	0	0	1	**3**		0	0	0	1	1	0	1	0	**3**		0	1	0	0
T9	1	0	0	1	**3**		0	0	0	0	0	1	1	0	**2**		0	0	0	0
Tenerife	**7**	**0**	**4**	**7**	**3.00**	**4**	**1**	**1**	**0**	**7**	**3**	**4**	**5**	**0**	**2.33**	**6**	**0**	**7**	**0**	**4**
M1	0	1	0	0	**1**		0	0	0	0	0	0	0	0	**0**		1	0	0	0
M2	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**		1	0	0	0
M3	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**		0	0	1	0
M4	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**		0	0	1	0
M5	0	1	0	0	**1**		0	0	0	0	0	0	0	1	**1**		1	0	1	0
M6	0	1	0	0	**2**		0	0	0	0	0	0	0	0	**0**		0	0	1	0
M7	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**		1	0	1	0
M8	0	1	0	0	**2**		0	0	0	0	0	0	0	0	**0**		0	0	0	0
M9	0	1	0	0	**1**		0	0	0	0	0	0	0	0	**0**		1	0	0	0
M10	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**		1	0	0	0
M11	0	1	0	0	**2**		0	0	0	0	0	0	0	0	**0**		1	0	0	0
Madeira	**0**	**11**	**0**	**0**	**1.73**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0.55**	**1**	**7**	**0**	**5**	**0**

Site	*Physa acuta*	*Pisidium casertanum*	*Pseudosuccinea columella*	**Mollusca species richness**	**Total island spp. richness**	*Anax imperator*	*Crocothemis erythraea*	early instars	*Hemianax ephippiger*	*Ischnura saharensis*	*Orthetrum chrysostigma*	*Sympetrum nigrifemur*	*Trithemis arteriosa*	*Zygonax torrida*	**Odonata species richness**	**Total island spp. richness**	*Agapetus adejensis*	*Hydropsyche maderensis*	*Hydropsyche sp.*	*Hydroptila* spp.
P1	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	0	0	0
P2	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	0	0	0
P3	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
P4	0	0	0	**0**		0	0	0	0	0	0	0	0	0	**0**		1	0	0	0
P5	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	0	0	0
P6	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
P7	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
P8	0	0	0	**0**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
P9	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
P10	0	0	0	**0**		0	0	0	0	0	0	0	0	0	**0**		0	0	0	1
P11	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
P12	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	1
La Palma	**0**	**0**	**0**	**0.75**	**1**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0.58**	**1**	**1**	**0**	**0**	**8**
G1	1	0	0	**2**		0	0	0	0	0	0	0	0	0	**0**		1	0	1	0
G2	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	0	1	0
G3	0	1	0	**2**		0	0	0	0	0	0	0	0	0	**0**		1	0	0	0
G4	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		1	0	0	0
G5	1	1	0	**3**		0	0	0	0	0	0	0	0	0	**0**		1	0	1	1
G6	0	1	0	**1**		0	0	0	0	0	0	0	0	0	**0**		1	0	0	0
G7	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		1	0	0	0
G8	1	0	0	**3**		0	0	0	0	0	1	1	0	0	**2**		0	0	0	1
G9	1	1	0	**4**		0	0	0	0	0	0	0	0	0	**0**		0	0	0	0
G10	1	1	0	**4**		0	0	0	0	0	0	0	0	0	**0**		1	0	0	0
La Gomera	**5**	**5**	**0**	**2.20**	**4**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0.20**	**2**	**7**	**0**	**3**	**2**
T1	1	0	1	**4**		1	1	1	0	1	1	1	1	0	**7**		0	0	1	1
T2	0	1	0	**2**		0	0	0	0	0	0	1	0	0	**1**		0	0	1	0
T3	1	0	1	**4**		1	0	1	1	0	1	1	1	1	**7**		0	0	1	1
T4	1	1	1	**5**		1	0	1	0	0	1	1	1	0	**5**		0	0	1	1
T5	1	0	1	**4**		0	0	0	0	0	1	1	0	1	**3**		1	0	1	1
T6	0	0	0	**0**		0	0	1	0	0	0	0	0	0	**1**		0	0	0	0
T7	0	0	0	**1**		0	0	0	0	0	0	1	0	0	**1**		0	0	0	1
T8	0	0	0	**1**		1	0	1	0	0	0	0	0	0	**2**		1	0	0	1
T9	0	0	0	**0**		0	0	0	0	0	0	0	0	0	**0**		0	0	0	1
Tenerife	**4**	**2**	**4**	**2.33**	**5**	**4**	**1**	**5**	**1**	**1**	**4**	**6**	**3**	**2**	**3.00**	**9**	**2**	**0**	**5**	**7**
M1	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M2	0	0	0	**1**		0	0	1	0	0	0	0	0	0	**1**		0	1	0	1
M3	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M4	0	1	0	**2**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M5	0	0	0	**2**		0	0	0	0	0	0	1	0	0	**1**		0	1	0	1
M6	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M7	0	0	0	**2**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M8	0	0	0	**0**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M9	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	0
M10	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
M11	0	0	0	**1**		0	0	0	0	0	0	0	0	0	**0**		0	1	0	1
Madeira	**0**	**1**	**0**	**1.18**	**3**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0.18**	**2**	**0**	**11**	**0**	**10**

Site	*Lepidostoma tenerifensis*	*Limnephilus nybomi*	*Mesophylax aspersus*	*Mesophylax oblitus*	*Oecetis* sp.	*Orthotrichia* spp.	*Oxyethira* spp.	*Oxyethira spinosella*	*Polycentropus flavostictus*	*Polycentropus tenerifensis*	*Stactobia* spp.	*Synagapetus* spp.	*Tinodes canariensis*	*Tinodes* spp.	*Wormaldia taganananа*	**Trichoptera spp. richness**	**Total island spp. richness**	**Overall island richness**
P1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**		4
P2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**		5
P3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**2**		11
P4	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	**4**		8
P5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**1**		6
P6	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**2**		14
P7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		12
P8	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	**3**		14
P9	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	**4**		15
P10	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	**3**		13
P11	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**2**		10
P12	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**2**		13
La Palma	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**5**	**0**	**0**	**2.17**	**5**	**25**
G1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	**6**		23
G2	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	**4**		19
G3	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	**4**		21
G4	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	**4**		17
G5	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	**6**		23
G6	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	**3**		10
G7	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	**4**		13
G8	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	**4**		23
G9	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	**2**		19
G10	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	**3**		17
La Gomera	**0**	**0**	**10**	**0**	**6**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**8**	**4.00**	**8**	**38**
T1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	**5**		40
T2	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	**5**		19
T3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	**4**		32
T4	0	0	1	0	0	1	1	0	0	0	0	0	1	0	1	**7**		29
T5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**4**		29
T6	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	**2**		11
T7	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	**4**		23
T8	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	**7**		27
T9	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	**3**		16
Tenerife	**1**	**0**	**7**	**0**	**2**	**2**	**6**	**0**	**0**	**2**	**1**	**0**	**3**	**0**	**3**	**4.56**	**12**	**61**
M1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**3**		5
M2	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	**7**		17
M3	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	**6**		12
M4	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	**5**		10
M5	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	**4**		9
M6	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	**5**		9
M7	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	**7**		15
M8	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	**5**		9
M9	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	**3**		5
M10	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	**4**		10
M11	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	**5**		9
Madeira	**0**	**5**	**0**	**4**	**0**	**0**	**0**	**3**	**4**	**0**	**4**	**2**	**0**	**11**	**0**	**4.91**	**9**	**23**

Appendix 3.2 Species presence/absence records for 42 Macaronesian streams arranged by land use type. L: *laurisilva*; P: pine forest; D: deforested land. Including: number of site records per species per land use type; number of species per group (*e.g.* order) per site and per land use type; mean number of species per group per site averaged for each land use type; and total number of species per site and per land use type.

Land Use	Site	*Agabus biguttatus*	*Agabus maderensis*	*Agabus nebulosus*	*Agabus wollastoni*	*Anacaena haemorrhoa*	*Bidessus minutissimus*	*Chaetarthria simillis*	*Coelostoma hispanicum*	*Cyphon gracilicornis*	*Dryops gracilis*	*Dryops luridus*	*Enochrus politus*	*Graptodytes delectus*	*Gyrinus dejeani*	*Gyrinus urinator*	*Haliplus lineaticollis*	*Helochares lividus*	*Hydraena serricollis*	*Hydrochus quadricollis*	*Hydroporus discretus*	*Hydroporus lucasi*	*Laccobius canariensis*
1	P1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1	P2	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
1	P3	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
1	P10	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
1	P11	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1	G1	1	0	0	0	1	0	1	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0
1	G2	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0
1	G3	1	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	1	0	1	0	1
1	G4	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1
1	G5	1	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	1	0	1	0	0
1	G6	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1	G7	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
1	G9	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	1
1	T2	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0
1	M1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1	M8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	M10	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1	M11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	P/A	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**0**	**1**	**0**	**1**	**0**	**1**
2	P4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	P5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	P6	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
2	P7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	P8	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
2	P9	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
2	P12	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
2	T5	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0	1
2	T6	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
2	T7	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1
2	T8	1	0	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1
2	T9	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
	P/A	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**
3	G8	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	0	1	0	1
3	G10	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0
3	T1	1	0	0	0	1	0	1	1	0	1	0	1	0	1	1	1	1	0	1	0	0	1
3	T3	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	1	0	1
3	T4	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1
3	M6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P/A	**1**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**

Land Use	Site	*Laccobius gracilis*	*Laccophilus hyalinus*	*Limnebius gracilipes*	*Limnebius similis*	*Limnebius grandicollis*	*Meladema coriacea*	*Meladema imbricata*	*Meladema lanio*	*Nebrioporus canariensis*	*Nebrioporus dubius*	*Ochthebius quadrifoveolatus*	*Ochthebius rugulosus*	**Coleoptera species richness**	**Mean stream richness**	*Chaetogamm. chaetocerus*	**Amphipoda species richness**	**Mean stream richness**
1	P1	0	0	0	0	0	0	0	0	0	0	0	0	2		0	0	
1	P2	0	0	0	0	0	0	0	0	0	0	0	0	3		0	0	
1	P3	0	0	0	0	0	0	0	0	1	0	0	0	4		0	0	
1	P10	0	0	1	0	0	0	0	0	1	0	0	0	6		0	0	
1	P11	0	0	0	0	0	0	0	0	1	0	0	0	3		0	0	
1	G1	0	0	1	0	0	0	1	0	0	0	0	0	10		1	1	
1	G2	0	0	1	0	0	0	0	0	1	0	0	0	9		1	1	
1	G3	0	0	1	0	0	0	0	0	1	0	0	0	11		0	0	
1	G4	0	0	0	1	0	0	1	0	1	0	0	0	9		0	0	
1	G5	0	0	1	0	0	0	0	0	1	0	0	0	9		0	0	
1	G6	0	0	0	0	0	0	0	0	1	0	0	0	5		0	0	
1	G7	0	0	0	0	0	0	0	0	0	0	0	0	6		0	0	
1	G9	0	0	1	0	0	0	0	0	1	0	0	0	9		0	0	
1	T2	0	0	1	0	0	0	0	0	1	0	0	0	8		0	0	
1	M1	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	
1	M2	0	0	0	0	1	0	0	1	0	1	0	0	5		0	0	
1	M3	0	0	0	0	0	0	0	1	0	0	0	0	2		0	0	
1	M4	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	
1	M5	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	
1	M7	0	0	0	0	0	0	0	1	0	1	0	0	3		0	0	
1	M8	0	0	0	0	0	0	0	1	0	1	0	0	2		0	0	
1	M9	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	
1	M10	0	0	0	0	0	0	0	0	0	1	0	0	2		0	0	
1	M11	0	0	0	0	0	0	0	0	0	0	0	0	1		0	0	
	P/A	0	0	1	1	1	0	1	1	1	1	0	0	22	4.54	1	1	0.08
2	P4	0	0	0	0	0	0	0	0	1	0	0	0	2		0	0	
2	P5	0	0	0	0	0	0	0	0	1	0	0	0	3		0	0	
2	P6	0	0	1	0	0	0	0	0	1	0	1	0	7		0	0	
2	P7	0	0	1	0	0	0	0	0	1	0	1	0	5		0	0	
2	P8	1	0	1	0	0	0	0	0	1	0	1	0	9		0	0	
2	P9	0	0	1	0	0	0	0	0	1	0	1	0	8		0	0	
2	P12	0	0	1	0	0	0	0	0	1	0	0	0	6		0	0	
2	T5	0	1	1	0	0	0	0	0	1	0	1	1	12		0	0	
2	T6	0	0	0	0	0	0	0	0	1	0	1	1	6		0	0	
2	T7	0	0	1	0	0	1	1	0	1	0	1	0	11		0	0	
2	T8	0	0	1	0	0	1	0	0	1	0	1	1	11		0	0	
2	T9	0	0	0	0	0	0	0	0	1	0	1	1	8		0	0	
	P/A	1	1	1	0	0	1	1	0	1	0	1	1	21	7.33	0	0	0.00
3	G8	0	1	0	0	0	0	0	0	1	0	0	0	10		0	0	
3	G10	0	0	1	0	0	0	0	0	0	0	0	0	6		0	0	
3	T1	0	1	1	0	0	0	0	0	1	0	1	0	16		0	0	
3	T3	0	0	1	0	0	1	0	0	1	0	0	0	10		0	0	
3	T4	0	0	1	0	0	0	0	0	1	0	1	0	7		0	0	
3	M6	0	0	0	0	0	0	0	1	0	0	0	0	1		0	0	
	P/A	0	1	1	0	0	1	0	1	1	0	1	0	22	8.33	0	0	0.00

Land Use	Site	*Baetis canariensis*	*Baetis pseudorhodani*	*Baetis pseudo./nigr.*	*Baetis rhodani*	*Caenis luctuosa*	*Cloeon* sp.	**Ephem. species richness**	**Mean stream richness**	*Corixidae* sp. nymph	*Gerris thoracicus*	*Hebrus pusillus*	*Hydrometra stagnorum*	*Microvelia gracillima*	*Notonecta canariensis*	*Velia lindbergi*	*Velia maderensis*	**Hemiptera species richness**	**Mean stream richness**
1	P1	0	0	0	0	0	0	**0**		0	0	0	0	0	0	0	0	**0**	
1	P2	0	0	0	0	0	0	**0**		0	0	0	0	0	0	1	0	**1**	
1	P3	1	0	0	0	0	0	**1**		0	0	0	1	0	0	1	0	**2**	
1	P10	1	0	1	0	0	1	**3**		0	0	0	0	0	0	1	0	**1**	
1	P11	1	0	0	0	0	0	**1**		0	0	0	0	1	0	1	0	**2**	
1	G1	1	0	1	0	0	0	**2**		0	0	0	0	1	0	1	0	**2**	
1	G2	1	0	1	0	0	0	**2**		0	0	0	0	1	0	1	0	**2**	
1	G3	1	0	1	0	0	0	**2**		0	0	0	0	1	0	1	0	**2**	
1	G4	1	0	1	0	0	0	**2**		0	0	0	0	0	0	1	0	**1**	
1	G5	1	0	1	0	0	0	**2**		0	0	0	1	1	0	1	0	**3**	
1	G6	0	0	0	0	0	0	**0**		0	0	0	0	0	0	1	0	**1**	
1	G7	0	0	0	0	0	0	**0**		0	0	0	0	1	0	1	0	**2**	
1	G9	1	0	0	0	0	0	**1**		0	0	0	1	1	0	1	0	**3**	
1	T2	1	0	0	0	0	0	**1**		0	0	0	0	1	0	1	0	**2**	
1	M1	0	0	0	1	0	0	**1**		0	0	0	0	0	0	0	0	**0**	
1	M2	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**	
1	M3	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**	
1	M4	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**	
1	M5	0	0	0	1	0	0	**1**		0	0	0	0	0	0	0	1	**1**	
1	M7	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**	
1	M8	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	0	**0**	
1	M9	0	0	0	1	0	0	**1**		0	0	0	0	0	0	0	0	**0**	
1	M10	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	1	**1**	
1	M11	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	0	**0**	
	P/A	**1**	**1**	**1**	**1**	**0**	**1**	**5**	**1.42**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**4**	**1.25**
2	P4	1	0	1	0	0	0	**2**		0	0	0	0	0	0	0	0	**0**	
2	P5	0	0	0	0	0	0	**0**		0	0	0	0	0	0	1	0	**1**	
2	P6	1	0	1	0	0	0	**2**		0	0	0	1	0	0	0	0	**1**	
2	P7	1	0	1	0	0	0	**2**		0	0	0	1	0	0	0	0	**1**	
2	P8	1	0	0	0	0	0	**1**		0	0	0	0	0	0	0	0	**0**	
2	P9	1	0	0	0	0	0	**1**		0	0	0	0	0	0	0	0	**0**	
2	P12	1	0	1	0	0	0	**2**		0	0	0	0	0	0	1	0	**1**	
2	T5	1	0	1	0	1	1	**4**		0	0	0	1	0	1	0	0	**2**	
2	T6	1	0	0	0	0	0	**1**		0	0	0	1	0	0	0	0	**1**	
2	T7	1	0	1	0	1	1	**4**		0	0	0	1	0	1	0	0	**2**	
2	T8	1	0	1	0	0	1	**3**		0	0	0	1	1	0	1	0	**3**	
2	T9	1	0	1	0	0	1	**3**		0	0	0	0	0	1	1	0	**2**	
	P/A	**1**	**0**	**1**	**0**	**1**	**1**	**4**	**2.08**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**0**	**4**	**1.17**
3	G8	1	0	0	0	0	1	**2**		0	0	0	1	0	0	1	0	**2**	
3	G10	0	0	0	0	0	0	**0**		0	0	1	1	1	0	1	0	**4**	
3	T1	1	0	1	0	1	1	**4**		1	1	0	1	0	0	1	0	**4**	
3	T3	1	0	1	0	0	1	**3**		0	0	0	1	1	1	1	0	**4**	
3	T4	1	0	1	0	1	1	**4**		0	0	0	1	0	0	0	0	**1**	
3	M6	0	1	0	1	0	0	**2**		0	0	0	0	0	0	0	0	**0**	
	P/A	**1**	**1**	**1**	**1**	**1**	**1**	**6**	**2.50**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**7**	**2.50**

Land Use	Site	*Ancylus fluviatilis*	*Ancylus striatus*	*Gyraulus parvus*	*Lymnaea truncatula*	*Physa acuta*	*Pisidium casertanum*	*Pseudosuccinea columella*	**Mollusca species richness**	**Mean stream richness**	*Anax imperator*	*Crocothemis erythraea*	early instars	*Hemianax ephippiger*	*Ischnura saharensis*	*Orthetrum chrysostigma*	*Sympetrum nigrifemur*	*Trithemis arteriosa*	*Zygonax torrida*	**Odonata species richness**	**Mean stream richness**
1	P1	0	1	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	P2	0	1	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	P3	0	1	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
1	P10	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	
1	P11	0	1	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
1	G1	0	1	0	0	1	0	0	2		0	0	0	0	0	0	0	0	0	0	
1	G2	0	1	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	G3	0	1	0	0	0	1	0	2		0	0	0	0	0	0	0	0	0	0	
1	G4	0	1	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	G5	0	1	0	0	1	1	0	3		0	0	0	0	0	0	0	0	0	0	
1	G6	0	0	0	0	0	1	0	1		0	0	0	0	0	0	0	0	0	0	
1	G7	0	1	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	G9	0	1	0	1	1	1	0	4		0	0	0	0	0	0	0	0	0	0	
1	T2	0	1	0	0	0	1	0	2		0	0	0	0	0	0	1	0	0	1	
1	M1	1	0	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	M2	1	0	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
1	M3	0	0	1	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	M4	0	0	1	0	0	1	0	2		0	0	0	0	0	0	0	0	0	0	
1	M5	1	0	1	0	0	0	0	2		0	0	0	0	0	0	1	0	0	1	
1	M7	1	0	1	0	0	0	0	2		0	0	0	0	0	0	0	0	0	0	
1	M8	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	
1	M9	1	0	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	M10	1	0	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
1	M11	1	0	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
	P/A	1	1	1	1	1	1	0	6	1.38	0	0	1	0	0	0	1	0	0	2	0.21
2	P4	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	
2	P5	0	1	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
2	P6	0	1	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
2	P7	0	1	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
2	P8	0	0	0	0	0	0	0	0		0	0	1	0	0	0	0	0	0	1	
2	P9	0	1	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
2	P12	0	1	0	0	0	0	0	1		0	0	1	0	0	0	0	0	0	1	
2	T5	0	1	0	1	1	0	1	4		0	0	0	0	0	1	1	0	1	3	
2	T6	0	0	0	0	0	0	0	0		0	0	1	0	0	0	0	0	0	1	
2	T7	0	1	0	0	0	0	0	1		0	0	0	0	0	0	1	0	0	1	
2	T8	0	1	0	0	0	0	0	1		1	0	1	0	0	0	0	0	0	2	
2	T9	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	
	P/A	0	1	0	1	1	0	1	4	0.92	1	0	1	0	0	1	1	0	1	5	1.00
3	G8	0	1	0	1	1	0	0	3		0	0	0	0	0	1	1	0	0	2	
3	G10	0	1	0	1	1	1	0	4		0	0	0	0	0	0	0	0	0	0	
3	T1	0	1	0	1	1	0	1	4		1	1	1	0	1	1	1	1	0	7	
3	T3	0	1	0	1	1	0	1	4		1	0	1	1	0	1	1	1	1	7	
3	T4	0	1	0	1	1	1	1	5		1	0	1	0	0	1	1	1	0	5	
3	M6	0	0	1	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
	P/A	0	1	1	1	1	1	1	6	3.50	1	1	1	1	1	1	1	1	1	9	3.50

Land Use	Site	*Agapetus adejensis*	*Hydropsyche maderensis*	*Hydropsyche* sp.	*Hydroptila* spp.	*Lepidostoma tenerifensis*	*Limnephilus nybomi*	*Mesophylax aspersus*	*Mesophylax oblitus*	*Oecetis* sp.	*Orthotrichia* spp.	*Oxyethira* spp.	*Oxyethira spinosella*	*Polycentropus flavostictus*	*Polycentropus tenerifensis*	*Stactobia* spp.	*Synagapetus* spp.	*Tinodes canariensis*	*Tinodes* spp.	*Wormaldia taganananana*	Trichoptera species richnes	Mean stream richness	Total stream richness
1	P1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1		4
1	P2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		5
1	P3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		11
1	P10	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3		13
1	P11	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		10
1	G1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	6		23
1	G2	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	4		19
1	G3	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	4		21
1	G4	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	4		17
1	G5	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	6		23
1	G6	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	3		10
1	G7	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	4		13
1	G9	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2		19
1	T2	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	5		19
1	M1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3		5
1	M2	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	7		17
1	M3	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	6		12
1	M4	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	5		10
1	M5	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	4		9
1	M7	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	7		15
1	M8	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	5		9
1	M9	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	3		5
1	M10	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	4		10
1	M11	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	5		9
	P/A	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	16	3.96	56
2	P4	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	4		8
2	P5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1		6
2	P6	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		14
2	P7	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		12
2	P8	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3		14
2	P9	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	4		15
2	P12	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		13
2	T5	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4		29
2	T6	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2		11
2	T7	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	4		23
2	T8	1	0	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	7		27
2	T9	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3		16
	P/A	1	0	1	1	0	0	1	0	1	0	1	0	0	1	1	0	1	0	0	9	3.17	47
3	G8	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	4		23
3	G10	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	3		17
3	T1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	5		40
3	T3	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	4		32
3	T4	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	1	7		29
3	M6	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	5		9
	P/A	1	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	1	12	4.67	62

Appendix 3.3 Species abundance records for 42 Macaronesian streams. Values are mean abundance in five replicate Surber samples. Species not found in Surber samples are omitted.

Site	*Agabus biguttatus*	*Agabus maderensis*	*Agabus nebulosus*	*Agabus wollastoni*	*Anacaena haemorrhoa*	*Bidessus minutissimus*	*Chaetarthria similis*	*Coelostoma hispanicum*	*Cyphon gracilicornis*	*Dryops gracilis*	*Dryops luridus*	*Enochrus politus*	*Graptodytes delectus*	*Gyrinus dejeani*	*Gyrinus urinator*	*Haliplus lineaticollis*
P1	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00
P2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00
P4	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P7	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
P9	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00	0.00
P10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P12	1.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G1	0.17	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00
G2	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.80	0.00	0.00	0.00	0.00	0.00	0.00
G3	2.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00
G4	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00
G5	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.80	0.00	0.00	0.00	0.00	0.00	0.00
G6	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.25	0.00	0.00	0.00	0.00	0.00	0.00
G7	3.33	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	10.33	0.00	0.00	0.00	0.00	0.00	0.00
G8	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.20
G9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.20	0.00	0.00	0.00	0.00	0.00	0.00
G10	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	34.67	0.00	0.00	0.00	0.00	0.33	0.00
T1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	4.20	0.00	0.00	0.00	0.00	0.20	0.00
T2	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.00
T4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00
T5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	14.60	0.00	0.00	0.00	0.00	0.00	0.00
T7	1.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	0.00	0.00	0.00	0.00	0.00	0.00
T8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.00
T9	4.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00
M1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M2	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00
M8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
M9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00

Site	*Helochares lividus*	*Hydraena serricollis*	*Hydrochus quadricollis*	*Hydroporus discretus*	*Hydroporus lucasi*	*Laccobius canariensis*	*Laccobius gracilis*	*Laccophilus hyalinus*	*Limnebius gracilipes*	*Limnebius similis*	*Limnebius grandicollis*	*Melodema imbricata*	*Melodema lanio*	*Nebrioporus canariensis*	*Nebrioporus dubius*	*Ochthebius quadrifoveolatus*
P1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.20	0.00	0.00	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80
P9	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	1.20
P10	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00
P11	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00
P12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G2	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G4	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G6	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G7	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G8	0.00	0.40	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
G9	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G10	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T1	0.00	0.00	0.00	0.00	0.00	4.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.80	0.00	0.20
T2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.40	0.00	0.00
T4	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.40
T5	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.17	0.00	0.33
T6	0.00	0.00	0.00	0.00	0.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	2.20
T7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.20
T8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.20	0.00
M3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.20	0.00
M9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Site	*Ochthebius rugulosus*	*Chaetogammarus chaetocerus*	*Baetis canariensis*	*Baetis pseudorhodani*	*Baetis pseudo./nigrescens*	*Baetis rhodani*	*Caenis luctuosa*	*Cloeon* spp.	*Corixidae* spp.	*Gerris thoracicus*	*Hebrus pusillus*	*Hydrometra stagnorum*	*Microvelia gracillima*	*Notonecta canariensis*	*Velia lindbergi*
P1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
P3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P4	0.00	0.00	22.50	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P6	0.00	0.00	14.80	0.00	4.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P7	0.00	0.00	0.50	0.00	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P9	0.00	0.00	25.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P10	0.00	0.00	20.60	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P12	0.00	0.00	30.00	0.00	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G1	0.00	15.33	3.17	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.17
G2	0.00	0.40	0.60	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.00	1.20
G3	0.00	0.00	25.80	0.00	13.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.00	0.20
G4	0.00	0.00	16.20	0.00	7.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60
G5	0.00	0.00	2.60	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.60
G6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
G7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.67
G8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40
G9	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
G10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.33	0.33	0.00	0.17
T1	0.00	0.00	2.00	0.00	2.20	0.00	19.60	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
T2	0.00	0.00	5.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.40
T3	0.00	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.40	0.00	0.00
T4	0.00	0.00	0.80	0.00	3.40	0.00	0.20	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
T5	0.00	0.00	9.83	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T7	0.00	0.00	5.60	0.00	4.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	0.00	0.00	20.80	0.00	5.20	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.20
T9	0.00	0.00	8.67	0.00	2.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M1	0.00	0.00	0.00	0.17	0.00	82.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M2	0.00	0.00	0.00	0.20	0.00	23.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M3	0.00	0.00	0.00	2.80	0.00	30.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M4	0.00	0.00	0.00	2.00	0.00	16.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M5	0.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M6	0.00	0.00	0.00	5.00	0.00	35.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M7	0.00	0.00	0.00	0.40	0.00	31.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M8	0.00	0.00	0.00	0.80	0.00	50.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M9	0.00	0.00	0.00	0.00	0.00	17.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M10	0.00	0.00	0.00	0.80	0.00	46.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M11	0.00	0.00	0.00	3.60	0.00	85.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Site	*Velia maderensis*	*Ancylus fluviatilis*	*Ancylus striatus*	*Gyraulus parvus*	*Galba truncatula*	*Physella acuta*	*Pisidium casertanum*	*Pseudosuccinea columella*	*Anax imperator*	*Crocothermis erythraea*	early instars of Odonata	*Hemianax ephippiger*	*Ishnura saharensis*	*Orthetrum chrysostigma*	*Sympetrum nigrifemur*	*Trithemis arteriosa*
P1	0.00	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P2	0.00	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00
P4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P6	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.80	0.00	0.00	0.00	0.00	0.00
P9	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00
P10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00
P12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00
G1	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G2	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G3	0.00	0.00	1.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G4	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G5	0.00	0.00	1.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G6	0.00	0.00	0.00	0.00	0.00	0.00	3.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G7	0.00	0.00	2.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G8	0.00	0.00	16.60	0.00	1.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G9	0.00	0.00	2.20	0.00	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G10	0.00	0.00	1.17	0.00	5.50	1.33	17.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T1	0.00	0.00	11.20	0.00	1.00	5.40	0.00	6.00	0.00	0.00	0.80	0.00	0.00	3.00	0.00	0.00
T2	0.00	0.00	2.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	0.00	0.00	12.20	0.00	0.60	1.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.20
T4	0.00	0.00	28.60	0.00	0.20	3.20	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20
T5	0.00	0.00	0.50	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.00
T6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	0.00	0.00	0.00	0.00	0.00
T7	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M1	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M2	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M3	0.80	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M4	0.40	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M5	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M6	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M7	0.20	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M9	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M11	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Site	*Zygonax torrida*	*Agapetus adejensis*	*Hydropsyche maderensis*	*Hydropsyche* sp.	*Hydroptila* spp.	*Lepidostoma tenerifensis*	*Limnephilus nybomi*	*Mesophylax aspersus*	*Mesophylax oblitus*	*Oecetis* sp.	*Orthotrichia* spp.	*Oxyethira* spp.	*Oxyethira spinosella*	*Polycentropus flavostictus*	*Polycentropus tenerifensis*
P1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P3	0.00	0.00	0.00	0.00	26.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P4	0.00	4.75	0.00	0.00	0.00	0.00	0.00	12.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P6	0.00	0.00	0.00	0.00	74.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P7	0.00	0.00	0.00	0.00	43.00	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.00	0.00	0.00	35.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P9	0.00	0.00	0.00	0.00	153.60	0.00	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P10	0.00	0.00	0.00	0.00	0.20	0.00	0.00	1.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.00	0.00	0.00	2.25	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P12	0.00	0.00	0.00	0.00	3.75	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G1	0.00	2.50	0.00	0.17	0.00	0.00	0.00	1.17	0.00	0.33	0.00	0.00	0.00	0.00	0.00
G2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.20	0.00	2.00	0.00	0.00	0.00	0.00	0.00
G3	0.00	3.20	0.00	0.00	0.00	0.00	0.00	3.20	0.00	3.20	0.00	0.00	0.00	0.00	0.00
G4	0.00	0.20	0.00	0.00	0.00	0.00	0.00	1.80	0.00	2.20	0.00	0.00	0.00	0.00	0.00
G5	0.00	1.60	0.00	3.60	0.20	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
G8	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00
G10	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.00
T1	0.00	0.00	0.00	109.60	27.60	0.00	0.00	0.00	0.00	0.00	7.40	6.00	0.00	0.00	0.00
T2	0.00	0.00	0.00	0.60	0.00	3.40	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
T3	0.00	0.00	0.00	3.60	14.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	0.00	0.00	0.00	11.60	129.20	0.00	0.00	0.40	0.00	0.00	0.20	0.00	0.00	0.00	0.00
T5	0.33	0.33	0.00	6.67	17.83	0.00	0.00	3.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	10.80	0.00	0.00	0.00
T7	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.20
T8	0.00	0.40	0.00	0.00	2.40	0.00	0.00	2.40	0.00	2.20	0.00	0.20	0.00	0.00	0.00
T9	0.00	0.00	0.00	0.00	4.83	0.00	0.00	23.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M1	0.00	0.00	0.17	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M2	0.00	0.00	1.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00
M3	0.00	0.00	0.20	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
M4	0.00	0.00	21.20	0.00	0.20	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00
M5	0.00	0.00	4.80	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00
M6	0.00	0.00	15.80	0.00	16.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.20	0.00	0.00
M7	0.00	0.00	9.20	0.00	4.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00
M8	0.00	0.00	7.40	0.00	40.20	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00
M9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M10	0.00	0.00	1.40	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M11	0.00	0.00	0.20	0.00	2.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00

Site	*Stactobia* spp.	*Synagapetus* spp.	*Tinodes canariensis*	*Tinodes* spp.	*Wormaldia taganananana*
P1	0.00	0.00	0.20	0.00	0.00
P2	0.00	0.00	0.00	0.00	0.00
P3	0.00	0.00	0.00	0.00	0.00
P4	0.00	0.00	0.25	0.00	0.00
P6	0.00	0.00	0.00	0.00	0.00
P7	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.00	0.00	0.00	0.00
P9	0.20	0.00	0.00	0.00	0.00
P10	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.00	0.00	0.00	0.00
P12	0.00	0.00	0.00	0.00	0.00
G1	0.00	0.00	0.83	0.00	28.67
G2	0.00	0.00	0.00	0.00	2.40
G3	0.00	0.00	0.00	0.00	34.00
G4	0.00	0.00	0.00	0.00	11.20
G5	0.00	0.00	0.00	0.00	7.60
G6	0.00	0.00	0.00	0.00	0.00
G7	0.00	0.00	0.00	0.00	0.67
G8	0.00	0.00	0.00	0.00	0.00
G9	0.00	0.00	0.00	0.00	0.00
G10	0.00	0.00	0.00	0.00	0.00
T1	0.00	0.00	0.00	0.00	0.00
T2	0.00	0.00	0.00	0.00	0.80
T3	0.00	0.00	0.00	0.00	0.00
T4	0.00	0.00	2.60	0.00	4.00
T5	0.00	0.00	0.00	0.00	0.00
T6	0.00	0.00	0.00	0.00	0.00
T7	0.00	0.00	0.00	0.00	0.00
T8	0.00	0.00	0.20	0.00	0.00
T9	0.00	0.00	0.17	0.00	0.00
M1	0.00	0.00	0.00	0.67	0.00
M2	0.00	0.40	0.00	1.60	0.00
M3	0.00	0.00	0.00	1.00	0.00
M4	0.00	0.00	0.00	0.60	0.00
M5	0.20	0.00	0.00	2.00	0.00
M6	0.80	0.00	0.00	0.60	0.00
M7	0.00	0.20	0.00	3.20	0.00
M8	0.00	0.00	0.00	2.80	0.00
M9	0.60	0.00	0.00	2.80	0.00
M10	0.00	0.00	0.00	1.20	0.00
M11	0.20	0.00	0.00	8.00	0.00

Appendix 3.4 Family abundance records for 42 Macaronesian streams. Values are mean abundance in five replicate Surber samples. Families not found in Surber samples are omitted.

Site	Gammaridae	Dytiscidae	Hydrophilidae	Scirtidae	Dryopidae	Gyrinidae	Haliplidae	Hydraenidae	Baetidae	Caenidae	Hebridae	Hydrometridae	Veliidae	Ancylidae	Planorbidae
P1	0.00	0.00	0.20	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.00
P3	0.00	0.00	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P4	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	22.75	0.00	0.00	0.00	0.00	0.00	0.00
P6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.60	0.00	0.00	0.00	0.00	0.80	0.00
P7	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	2.75	0.00	0.00	0.00	0.00	0.00	0.00
P8	0.00	0.00	1.80	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P9	0.00	0.60	1.00	0.00	1.20	0.00	0.00	1.20	25.80	0.00	0.00	0.00	0.00	2.00	0.00
P10	0.00	0.20	0.00	0.00	0.20	0.00	0.00	0.20	22.60	0.00	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.25	0.50	0.00	0.00	0.00	0.00	0.25	0.00
P12	0.00	1.75	0.00	0.00	0.00	0.00	0.00	0.00	32.25	0.00	0.00	0.00	0.00	0.00	0.00
G1	15.33	0.17	0.17	0.00	1.50	0.00	0.00	0.00	5.67	0.00	0.00	0.00	1.50	2.00	0.00
G2	0.40	0.40	0.00	0.00	3.80	0.00	0.00	0.60	0.80	0.00	0.00	0.00	3.00	0.20	0.00
G3	0.00	2.80	0.00	0.00	1.80	0.00	0.00	0.00	39.40	0.00	0.00	0.00	2.00	1.60	0.00
G4	0.00	0.40	0.00	0.00	0.80	0.00	0.00	0.20	23.40	0.00	0.00	0.00	0.60	0.40	0.00
G5	0.00	0.20	0.00	0.00	3.80	0.00	0.00	0.00	3.60	0.00	0.00	0.00	1.40	1.00	0.00
G6	0.00	0.50	0.00	0.00	5.25	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50	0.00	0.00
G7	0.00	3.33	1.00	0.00	10.33	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.67	2.67	0.00
G8	0.00	1.20	0.00	0.00	3.40	0.00	0.20	0.40	0.00	0.00	0.00	0.00	0.40	16.60	0.00
G9	0.00	0.00	0.00	0.00	2.20	0.00	0.00	0.60	1.00	0.00	0.00	0.20	0.00	2.20	0.00
G10	0.00	0.17	0.00	0.17	34.67	0.33	0.00	0.50	0.00	0.00	0.17	0.33	0.50	1.17	0.00
T1	0.00	3.80	4.60	0.00	4.20	0.20	0.00	0.20	4.20	19.60	0.00	0.20	0.00	11.20	0.00
T2	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	5.40	0.00	0.00	0.00	0.80	2.00	0.00
T3	0.00	2.40	0.40	0.00	3.40	0.00	0.00	0.00	0.80	0.00	0.00	0.20	0.40	12.20	0.00
T4	0.00	1.00	0.40	0.00	3.00	0.00	0.00	0.40	4.20	0.20	0.00	0.20	0.00	28.60	0.00
T5	0.00	0.17	0.67	0.00	7.00	0.00	0.00	0.50	11.83	0.00	0.00	0.00	0.00	0.50	0.00
T6	0.00	0.40	6.00	0.00	14.60	0.00	0.00	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T7	0.00	1.40	0.00	0.00	1.40	0.00	0.00	0.20	10.40	0.00	0.00	0.00	0.00	0.40	0.00
T8	0.00	0.00	0.00	0.00	3.40	0.00	0.00	0.00	26.00	0.00	0.00	0.20	0.20	0.40	0.00
T9	0.00	4.33	0.00	0.00	1.67	0.00	0.00	0.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00
M1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	82.67	0.00	0.00	0.00	0.00	0.17	0.00
M2	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.20	23.40	0.00	0.00	0.00	0.00	0.20	0.00
M3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.00	0.00	0.00	0.00	0.80	0.00	0.60
M4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.20	0.00	0.00	0.00	0.40	0.00	0.00
M5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	0.00	1.80	0.00
M6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.40	0.00	0.00	0.00	0.00	0.00	0.40
M7	0.00	0.00	0.00	0.00	0.80	0.00	0.00	0.00	31.80	0.00	0.00	0.00	0.20	0.00	0.40
M8	0.00	0.40	0.00	0.00	0.20	0.00	0.00	0.00	51.60	0.00	0.00	0.00	0.00	0.00	0.00
M9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.60	0.00	0.00	0.00	0.00	2.00	0.00
M10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	47.40	0.00	0.00	0.00	0.00	0.00	0.00
M11	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	89.20	0.00	0.00	0.00	0.00	0.60	0.00

Site	Lymnaeidae	Physidae	Pisidiidae	Libellulidae	Glossosomatidae	Hydropsychidae	Hydroptilidae	Sericostomatidae	Limnephilidae	Leptoceridae	Polycentropodidae	Psychomyiidae	Philopotamidae	Simuliidae
P1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	21.86
P3	0.00	0.00	0.00	0.50	0.00	0.00	26.00	0.00	0.50	0.00	0.00	0.00	0.00	2.80
P4	0.00	0.00	0.00	0.00	4.75	0.00	0.00	0.00	12.75	0.00	0.00	0.25	0.00	1.00
P6	0.00	0.00	0.00	0.00	0.00	0.00	74.20	0.00	0.00	0.00	0.00	0.00	0.00	32.40
P7	0.00	0.00	0.00	0.75	0.00	0.00	43.00	0.00	1.50	0.00	0.00	0.00	0.00	62.60
P8	0.00	0.00	0.00	6.80	0.00	0.00	35.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80
P9	0.00	0.00	0.00	3.00	0.00	0.00	153.80	0.00	3.40	0.00	0.00	0.00	0.00	129.40
P10	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	1.60	0.00	0.00	0.00	0.00	38.60
P11	0.00	0.00	0.00	0.25	0.00	0.00	2.25	0.00	0.50	0.00	0.00	0.00	0.00	2.60
P12	0.00	0.00	0.00	2.00	0.00	0.00	3.75	0.00	0.25	0.00	0.00	0.00	0.00	56.80
G1	0.00	0.00	0.00	0.00	2.50	0.17	0.00	0.00	1.17	0.33	0.00	0.83	28.67	13.67
G2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.20	2.00	0.00	0.00	2.40	0.00
G3	0.00	0.00	0.00	0.00	3.20	0.00	0.00	0.00	3.20	3.20	0.00	0.00	34.00	4.80
G4	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	1.80	2.20	0.00	0.00	11.20	8.00
G5	0.00	0.20	0.00	0.00	1.60	3.60	0.20	0.00	1.00	0.00	0.00	0.00	7.60	5.20
G6	0.00	0.00	3.25	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.20
G7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.67	0.00
G8	1.40	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.60	0.00	0.00	0.00	0.00	17.40
G9	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.20	0.00	0.00	0.00	48.80
G10	5.50	1.33	17.83	0.00	0.50	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	3.17
T1	7.00	5.40	0.00	3.80	0.00	109.60	41.00	0.00	0.00	0.00	0.00	0.00	0.00	306.00
T2	0.00	0.00	0.40	0.00	0.00	0.60	0.00	3.40	0.00	0.20	0.00	0.00	0.80	2.20
T3	1.20	1.00	0.00	0.80	0.00	3.60	14.00	0.00	0.00	0.00	0.00	0.00	0.00	47.40
T4	0.40	3.20	0.00	1.00	0.00	11.60	129.40	0.00	0.40	0.00	0.00	2.60	4.00	122.60
T5	0.00	0.17	0.00	0.83	0.33	6.67	17.83	0.00	3.50	0.00	0.00	0.00	0.00	10.50
T6	0.00	0.00	0.00	1.40	0.00	0.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80
T7	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.40	0.00	0.20	0.00	0.00	5.40
T8	0.00	0.00	0.00	0.00	0.40	0.00	2.60	0.00	2.40	2.20	0.00	0.20	0.00	17.80
T9	0.00	0.00	0.00	0.00	0.00	0.00	4.83	0.00	23.83	0.00	0.00	0.17	0.00	0.50
M1	0.00	0.00	0.00	0.00	0.00	0.17	1.33	0.00	0.00	0.00	0.00	0.67	0.00	89.83
M2	0.00	0.00	0.00	0.00	0.40	1.00	0.40	0.00	0.00	0.00	2.00	1.60	0.00	23.60
M3	0.00	0.00	0.00	0.00	0.00	0.20	3.80	0.00	0.00	0.00	0.00	1.00	0.00	114.80
M4	0.00	0.00	0.20	0.00	0.00	21.20	0.20	0.00	1.80	0.00	0.60	0.60	0.00	80.20
M5	0.00	0.00	0.00	0.00	0.00	4.80	1.20	0.00	0.00	0.00	0.00	2.00	0.00	16.20
M6	0.00	0.00	0.00	0.00	0.00	15.80	21.00	0.00	0.00	0.00	0.00	0.60	0.00	28.80
M7	0.00	0.00	0.00	0.00	0.20	9.20	4.40	0.00	0.00	0.00	0.40	3.20	0.00	14.60
M8	0.00	0.00	0.00	0.00	0.00	7.40	40.20	0.00	0.20	0.00	0.80	2.80	0.00	17.00
M9	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	2.80	0.00	5.60
M10	0.00	0.00	0.00	0.00	0.00	1.40	0.20	0.00	0.00	0.00	0.00	1.20	0.00	3.80
M11	0.00	0.00	0.00	0.00	0.00	0.20	2.20	0.00	0.20	0.00	0.00	8.00	0.00	13.40

Site	Chironomidae	Dixidae	Psychodidae	Tipulidae	Muscidae	Stratiomyidae	Empididae	Dolichopodidae	Ceratopogonidae	Limoniidae	Tabanidae	Scatopsidae	Scathophagidae	Thaumaleidae	Chaoboridae	Ephydridae
P1	0.43	0.00	0.14	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P3	1.80	0.60	0.20	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P4	25.00	0.00	0.20	0.60	0.00	0.00	0.20	0.40	0.20	0.60	0.00	0.00	0.00	0.20	0.00	0.00
P6	53.20	0.20	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.40	0.00	0.00	0.00	0.00	0.00	0.00
P7	18.00	2.60	0.00	0.00	0.40	0.00	4.20	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.60
P8	7.80	0.40	0.80	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20
P9	75.00	3.20	0.20	0.00	0.00	0.00	1.60	0.00	0.40	0.20	0.20	0.00	0.00	0.00	0.00	0.00
P10	25.20	1.00	0.00	0.00	0.00	0.00	0.60	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P11	54.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	1.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P12	6.00	2.20	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G1	61.33	0.33	0.00	0.00	0.00	0.00	0.83	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
G2	317.20	3.40	0.00	0.00	0.20	0.00	1.40	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G3	120.20	0.20	0.80	0.20	0.20	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G4	19.00	0.20	0.80	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G5	167.20	1.00	38.60	0.00	0.00	0.00	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00
G6	5.40	0.60	1.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G7	2.40	0.60	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G8	59.60	4.40	0.20	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G9	3.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G10	22.17	0.00	0.00	0.17	1.33	0.00	0.00	0.17	0.17	0.00	0.00	0.00	0.00	0.67	0.00	0.00
T1	105.00	7.40	0.00	0.00	5.00	0.20	0.40	0.00	0.00	2.00	0.00	0.20	1.40	0.00	0.00	1.00
T2	15.60	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	20.00	5.80	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	90.40	1.40	0.00	0.00	3.80	0.40	0.00	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00	0.20
T5	51.17	0.00	1.17	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	80.80	0.40	0.00	1.00	0.20	0.00	0.60	0.00	2.80	0.20	0.00	0.00	0.00	0.00	0.00	0.00
T7	10.80	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	1.80	2.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	128.50	0.17	2.50	0.00	0.00	0.00	4.67	0.00	2.00	1.17	0.00	0.00	0.00	0.00	0.00	0.00
M1	17.67	0.67	0.17	0.00	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
M2	23.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
M3	66.60	3.20	0.20	0.00	0.00	0.00	1.60	0.20	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
M4	46.80	2.20	0.00	0.00	0.00	0.00	0.80	0.20	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00
M5	17.80	1.60	0.00	0.00	0.00	0.00	0.20	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00	0.60
M6	14.80	0.40	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
M7	37.60	3.40	0.00	0.20	0.00	0.00	1.80	0.20	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
M8	18.20	2.20	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M9	6.80	0.00	0.00	0.20	0.00	0.00	0.60	0.00	0.00	0.40	0.00	0.00	0.00	0.20	0.00	0.00
M10	15.60	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
M11	31.20	0.20	0.00	0.00	0.00	0.00	1.60	0.20	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 4.1 Species occupancy, median abundance and endemism. See below for explanation of coding used. Occupancy is proportion of streams occupied, using all sampling methods employed; abundance is median summed pool and riffle counts across streams occupied.

Species	Order	Occupancy	Abundance	Endemism	Dispersal
Agabus biguttatus	1	0.742	4	4	1
Agabus maderensis	1	0.143	2.5	1	1
Agabus nebulosus	1	0.182	1	4	1
Agabus wollastoni	1	0.355	1	1	1
Anacaena haemorrhoa	1	0.091	1	1	1
Bidessus minutissimus	1	0.032	1	4	1
Chaetarthria similis	1	0.129	5	4	1
Cyphon gracilicornis	1	0.226	1	1	1
Dryops gracilis	1	0.667	10.5	4	1
Dryops luridus	1	0.273	1	4	1
Enochrus politus	1	0.048	4	4	1
Graptodytes delectus	1	0.258	1.5	1	1
Gyrinus dejeani	1	0.032	5	4	1
Gyrinus urinator	1	0.323	2	4	1
Haliplus lineaticollis	1	0.129	7	4	1
Helochares lividus	1	0.065	1	4	1
Hydraena serricollis	1	0.405	3	1	1
Hydrochus grandicollis	1	0.065	1.5	4	1
Hydroporus discretus	1	0.387	2	4	1
Hydroporus lucasi	1	0.032	1	4	1
Laccobius canariensis	1	0.581	12	1	1
Laccophilus hyalinus	1	0.097	2	4	1
Limnebius gracilipes	1	0.613	1	1	1
Melademа lanio	1	0.455	5.5	1	1
Nebrioporus canariensis	1	0.839	12	1	1
Nebrioporus dubius	1	0.364	3.5	1	1
Ochthebius quadrifoveolatus	1	0.262	6	4	1
Ochthebius rugulosus	1	0.095	1	4	1
Baetis canariensis	2	0.806	35.5	1	1
Baetis pseudorhodani	2	0.548	3	1	1
Baetis psuedorh./nigrescens	2	0.727	2	4	1
Baetis rhodani	2	1.000	26	4	1
Caenis luctuosa	2	0.129	27	4	1
Cloeon sp.	2	0.290	30	1	1
Gerris thoracicus	3	0.032	5	4	1
Hydrometra stagnorum	3	0.452	1	4	1
Microvelia gracillima	3	0.355	3	4	1
Notonecta canariensis	3	0.129	1	1	1
Velia lindbergi	3	0.677	5	1	1
Velia maderensis	3	0.545	4.5	1	1
Ancylus fluviatilis	4	0.636	1	4	2

Species	Order	Occupancy	Abundance	Endemism	Dispersal
Ancylus striatus	4	0.806	1	1	2
Lymnaea truncatula	4	0.167	12	4	2
Gyraulus parvus	4	0.455	7	4	2
Physa acuta	4	0.214	13	4	2
Pisidium casertanum	4	0.190	18	4	2
Pseudosuccinea columella	4	0.129	20	4	2
Anax imperator	5	0.095	2	4	1
Crocothemis erythraea	5	0.032	13	4	1
Hemianax ephippiger	5	0.032	1	4	1
Ischnura saharensis	5	0.032	2	4	1
Orthetrum chrysostigma	5	0.161	2.5	4	1
Sympetrum nigrifemur	5	0.190	3	1	1
Trithemis arteriosa	5	0.097	1.5	4	1
Zygonax torrida	5	0.065	1	4	1
Agapetus adejensis	6	0.323	15	1	1
Hydropsyche maderensis	6	1.000	1	1	1
Hydropsyche sp.	6	0.258	9.5	4	1
Hydroptila spp.	6	0.643	42	4	1
Lepidostoma tenerifensis	6	0.032	311	1	1
Limnephilus nybomi	6	0.455	1	1	1
Mesophylax aspersus	6	0.871	40	4	1
Mesophylax oblitus	6	0.364	1	1	1
Oecetis sp.	6	0.258	14	1	1
Orthotrichia spp.	6	0.065	24	1	1
Oxyethira spinosella	6	0.273	7	1	1
Oxyethira spp.	6	0.258	5	1	1
Polycentropus flavostictus	6	0.364	3	1	1
Polycentropus tenerifensis	6	0.065	10.5	1	1
Stactobia spp.	6	0.167	1	1	1
Tinodes canariensis	6	0.323	2	1	1
Tinodes spp.	6	1.000	1.5	1	1
Wormaldia tagananana	6	0.355	22	1	1
Chaetogammarus chaetocerus	7	0.065	541.5	1	2

Key to Coding:

Group	1	Coleoptera
	2	Ephemeroptera
	3	Hemiptera
	4	Mollusca
	5	Odonata
	6	Trichoptera
	7	Amphipoda
Endemism	1	Endemic to Macaronesia
	4	Non-endemic
Dispersal	1	Active disperser
	2	Passive disperser

Appendix 6.1 General laboratory reagents used in electrophoresis. Catalogue numbers are from Sigma except where otherwise indicated.

Reagent	Cat. No.	Description	Storage
cis-Aconitic acid	A-3412		Solid, -20°C 10mg/ml, -20°C
Agarose	Helena 8201-03	Molecular biology grade	Room temp.
Albumin	A-2153	Bovine albumin, min. 96%	4°C
Arsenic acid	A-6576	Sodium salt, heptahydrate	Room temp. 6mg/ml, -20°C
ATP (adenosine 5'-triphosphate)	A-6144	Disodium salt, from equine muscle	Solid, -20°C 10mg/ml, 4°C
Boric acid	B-0252		Room temp.
Citric acid	C-0759	Anhydrous	Room temp.
o-Dianisidine	D-3252	Dihydrochloride, purified	4°C
DTT (DL-dithiothreitol)	D-0632	Min. 99%	4°C
EDTA (ethylenediaminetetraacetic acid)	E-5134	Disodium salt, dihydrate	Room temp.
Ethanol	BDH 437433T	Absolute ethanol	-20°C
Fast blue RR salt	F-0500		-20°C
Fast garnet GDC salt	Aldrich 20, 123-5		Room temp.
D-Fructose 1,6-diphosphate	F-4757		Solid, -20°C 10mg/ml, 4°C
D-Fructose 6-phosphate	F-3627	Disodium salt	Solid, -20°C 10mg/ml, 4°C
Fumaric acid	F-1506	Disodium salt	Solid, room temp. 50mg/ml, pH 8.0,-20°C
D-Glucose	G-8270		Solid, room temp. 10 mg/ml, -20°C
α-D-Glucose 1-phosphate	G-7000	Disodium salt, hydrate	Solid, -20°C 10mg/ml, 4°C
D-Glucose 6-phosphate	G-7250	Disodium salt, hydrate	Solid, -20°C 30mg/ml, -20°C
Glucose 6-phosphate dehydrogenase	G-5760	Type XXIII, from *Leuconostoc mesenteroides*	1 unit in 1μl, 4°C

Reagent	Cat. No.	Description	Storage
Glyceraldehyde 3-phosphate dehydrogenase	G-0763	From rabbit muscle	1 unit in 4µl, 4°C
DL-α-Glycerophosphate	G-6014	Disodium salt	4°C
Glycine	G-7126	Ammonia-free aminoacetic acid	Room temp.
Gly-Leu (glycine-leucine)	G-2002		Solid, -20°C 10mg/ml, -20°C
Hexokinase	H-5000	Type III from bakers yeast	Solid, -20°C 10mg/ml, -20°C
Hydrochloric acid	BDH 45002	30% hydrochloric acid	Room temp. 1M soln., 4°C 5M soln., 4°C
DL-Isocitric acid	I-1252	Trisodium salt	Room temp.
Isocitric dehydrogenase	I-2002	Type IV from porcine heart	1 unit in 20µl, 4°C
α-Lactic acid	L-1250	Synthetic syrup	Room temp.
Leu-Gly-Gly (leucine-glycine-glycine)	L-9750		Solid, -20°C 10mg/ml, -20°C
L-Leucine β-naphthylamide	L-0376	Hydrochloride	Solid, -20°C 10mg/ml, -20°C
Lithium hydroxide	L-4256	Monohydrate	Room temp.
Magnesium acetate	M-0631	Tetrahydrate	Solid, room temp. 0.25M soln., 4°C
Magnesium chloride	M-8266	Anhydrous	Solid, room temp. 30mg/ml, 4°C
Maleic acid	M-0375		Room temp.
DL-Malic acid	M-0875		Solid, room temp. 0.5M soln., pH 8.0, 4°C
Malic dehydrogenase	M-9004		6 units in 5µl, 4°C
D-Mannose 6-phosphate	M-8754	Barium salt	Solid, -20°C 10mg/ml, -20°C
2-Mercaptoethanol	M-3148		Room temp.
MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide)	M-2128		Solid, 4°C 10mg/ml, 4°C
NAD (β-nicotinamide adenosine dinucleotide)	N-7004	From yeast	Solid, -20°C 10mg/ml, -20°C

Reagent	Cat. No.	Description	Storage
NADP (β-nicotinamide adenine dinucleotide phosphate)	N-3886	Sodium salt	Solid, -20°C 1mg/ml, -20°C
α-Naphthyl acetate	N-8505		Solid, -20°C 10mg/ml, 4°C
β-Naphthyl acetate	N-6875		Solid, -20°C 10mg/ml, 4°C
α-Naphthyl butyrate	N-8000		Solid, -20°C 10mg/ml, -20°C
Peroxidase	P-8125	Type I from horseradish	Solid, -20°C 100mg/ml, 4°C
Phe-Pro (phenylalanine-proline)	P-6258		Solid, -20°C 10 mg/ml, -20°C
PEP (phospho(enol)pyruvate)	P-7127	Monopotassium salt	Solid, -20°C 10 mg/ml, -20°C
6-Phosphogluconic acid	P-7877	Trisodium salt, Grade IV	Solid, -20°C 10mg/ml, -20°C
Phosphoglucose isomerase	P-3381	Type III from bakers yeast	1 unit in 1µl, 4°C
PIPES (piperazine-N,N'-bis[2-ethanesulphonic acid])	P-6757		Room temp.
PMS (phenazine methosulphate)	P-9625		Solid, -20°C 2.5mg/ml, 4°C
Potassium chloride	P-4504	Anhydrous	Solid, room temp. 10mg/ml, -20°C
Potassium di-hydrogen phosphate	P-5379	Anhydrous	Room temp.
PVP (polyvinyl pyrrolidone)	Sigma PVP-40	Average molecular weight 40,000	Room temp.
Pyridoxal 5-phosphate	P-9255		-20°C
Snake venom	V-7000	From *Crotalus atrox* (Western Diamondback rattlesnake). Source of L-amino oxidase	Solid, -20°C 10mg/ml, -20°C
Sodium di-hydrogen orthophosphate	S-0751	Dihydrate	Room temp.
di-Sodium hydrogen orthophosphate	S-0876	Anhydrous	Room temp.
Sodium hydroxide	S-5881		Solid, room temp. 1M soln., 4°C 5M soln., 4°C
Sucrose	S-9378		Room temp.
Triosephosphate isomerase	T-2507	Type I from bakers yeast	0.5 units in 1µl, 4°C

Reagent	Cat. No.	Description	Storage
Triton X-100 (t-octylphenoxypolyethoxyethanol)		X-100	Room temp.
TRIZMA base (tris[hydroxymethyl]-aminomethane)	T-1503	Reagent grade	Room temp.

Appendix 6.2 Stock solutions used in electrophoresis. Buffer solutions taken from Richardson *et al.* (1986) and Peakall and Beattie (1991). PTP buffer from Dr. B. Wood, University of Birmingham (*pers. comm.*). Unless otherwise stated, stored at 4°C.

Function	Name	Molarity	pH	Composition
Grinding buffer for *Mesophylax aspersus* and *Wormaldia taganananа*	Basic homogenising buffer	0.5M	pH 8.0	To 100ml distilled water were added: 6.06g TRIZMA base 50µl 2-mercaptoethanol 2 drops Triton X-100 5M dilute hydrochloric acid to correct pH
Grinding buffer for *Wormaldia taganananа*	PTP homogenising buffer		pH 6.8	0.756g PIPES 3ml Triton X-100 Made up to 40ml with distilled water 5M sodium hydroxide added to pH 5.5, then 250mg pyridoxole 5-phosphate 5M sodium hydroxide to correct pH Made up to 50ml with distilled water
Grinding buffer for *Wormaldia taganananа* and *Ancylus striatus*	Peakall and Beattie (1991) homogenising buffer			145.5ml tris buffer, 1M, pH 8.0 18.2g sucrose 7.3mg PVP 180mg EDTA 90mg albumin 90mg NAD 90mg NADP 0.25M tris added to pH 7.0, then 182mg DTT Made up to 200ml with distilled water Stored in 1ml aliquots at -20°C
Running buffer	Citrate-phosphate (CP) buffer	0.01M	pH 6.4	1.42g di-sodium hydrogen phosphate 0.53g citric acid to correct pH Made up to 1l with distilled water
Running buffer	Phosphate (P) buffer	0.02M	pH 7.0	1.99g di-sodium hydrogen orthophosphate 1.31g sodium di-hydrogen orthophosphate to correct pH Made up to 1l with distilled water
Running buffer	Tris-borate (TB) buffer	0.13M	pH 8.9	15.74g TRIZMA base 0.82g EDTA 0.24g sodium hydroxide 4.41g boric acid to correct pH Made up to 1l with distilled water
Running buffer	Tris-citrate (TC) 7.6 buffer	0.04M	pH 7.6	4.485g TRIZMA base 5.25g citric acid to correct pH Made up to 1.15l with distilled water
Running buffer	Tris-citrate (TC) 8.2 buffer	0.1M	pH 8.2	12.11g TRIZMA base 3.99g citric acid to correct pH Made up to 1l with distilled water

Function	Name	Molarity	pH	Composition
Running buffer	Tris-glycine (TG) buffer	0.025M	pH 8.5	3.03g TRIZMA base 14.4g glycine 1M dilute hydrochloric acid to correct pH Made up to 1l with distilled water
Running buffer	Tris-malate (TM) buffer	0.1M	pH 7.4	12.11g TRIZMA base 0.37g EDTA 0.095g magnesium chloride 5.00g maleic acid to correct pH Made up to 1l with distilled water
Buffer used in majority of stains	Tris buffer 8.0	1M	pH 8.0	12.11g TRIZMA base 5M dilute hydrochloric acid to correct pH Made up to 100ml with distilled water
Buffer used in several stains	Phosphate buffer	0.1M	pH 7.0	1.36g potassium di-hydrogen phosphate 1M sodium hydroxide to correct pH Made up to 100ml with distilled water
Buffer used in aldolase stain	Tris buffer 7.4	0.1M	pH 7.4	1.211g TRIZMA base 5M dilute hydrochloric acid to correct pH Made up to 100ml with distilled water
Substrate for L-lactate dehydrogenase	Lithium lactate	1M	pH 8.0	13.5ml α-lactic acid Lithium hydroxide to correct pH Made up to 150ml with distilled water

Appendix 6.3 Specific staining methods for *Mesophylax aspersus*. Modified from Richardson *et al.* (1986), Hillis and Moritz (1990) and Jackson and Resh (1991). Enzymes identified by standard abbreviations and IUBNC (1984) Enzyme Commission numbers given. Stains suspended in agarose (720mg in 50ml of water at 60°C). Bands mobile from cathode to anode, except where stated otherwise. Incubation at 37°C. No successful method was established for ACON, ADH, ALD, LDH and 6PG.

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
EST Esterase E.C. 3.1.1._	2ml phosphate buffer 200µl α-naphthyl acetate 5mg fast blue RR salt	TG	20min	40min
FUM Fumarate hydratase E.C. 4.2.1.2	2ml tris buffer 1ml NAD 200µl fumaric acid 5µl malic dehydrogenase 100µl MTT 100µl PMS	CP	20min from anode	30min
G6P Glucose 6-phosphate dehydrogenase E.C. 1.1.1.49	1ml tris buffer 1ml NADP 500µl D-glucose 6-phosphate 300µl magnesium chloride 200µl PMS 200µl MTT	TC 7.6	20min	30min
GPI Glucose 6-phosphate isomerase E.C. 5.3.1.9	1ml tris buffer 1.5ml NADP 1ml D-fructose 6-phosphate 400µl magnesium acetate 10µl glucose 6-phosphate dehydrogenase 100µl PMS 100µl MTT	TC 8.2	40min	5min
IDH Isocitrate dehydrogenase E.C. 1.1.1.42	600µl tris buffer 1.5ml NADP 1ml distilled water 500µl magnesium chloride 50mg isocitric acid 200µl PMS 200µl MTT	TC 7.6	20min	5min
LAP Leucine aminopeptidase E.C. 3.4._._	5ml phosphate buffer 100µl magnesium chloride 10µl L-leucine-β-naphthylamide 6mg fast garnet GDC salt	TC 7.6	20min	1h

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
MDH NAD-dependent malate dehydrogenase E.C. 1.1.1.37	400µl tris buffer 1ml NAD 700µl malic acid 200µl PMS 200µl MTT	TC 7.6	30min	30min
MEN NADP-dependent malate dehydrogenase E.C. 1.1.1.40	800µl tris buffer 1ml NADP 1ml malic acid 100µl magnesium chloride 200µl PMS 200µl MTT	TG	40min	30min
MPI Mannose 6-phosphate isomerase E.C. 5.3.1.8	1ml tris buffer 1ml NADP 1ml D-mannose 6-phosphate 200µl magnesium chloride 14µl glucose 6-phosphate dehydrogenase 10µl phosphoglucose isomerase 200µl PMS 200µl MTT	TC 7.6	20min	30min
PEP B Leucine-glycine- glycine peptidase E.C. 3.4._._	2ml tris buffer 500µl leu-gly-gly 200µl snake venom 100µl peroxidase 100µl magnesium chloride 8mg O-dianisidine	P	30min	2h
PEP C Glycyl-L-leucine peptidase E.C. 3.4._._	2ml tris buffer 1ml gly-leu 200µl snake venom 100µl peroxidase 100µl magnesium chloride 8mg O-dianisidine	CP	30min	30min
PEP D Proline dipeptidase E.C. 3.4._._	2ml tris buffer 1ml phe-pro 400µl snake venom 200µl peroxidase 200µl magnesium chloride 16mg O-dianisidine	TC 8.2	30min	30min
PGM Phosphogluco- mutase 5.4.2.2	400µl tris buffer 1ml NADP 500µl magnesium acetate 400µl α-D-glucose 1-phosphate 10µl glucose 6-phosphate dehydrogenase 200µl PMS 200µl MTT	TC 7.6	20min	20min

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
PYK Pyruvate kinase E.C. 2.7.1.40	2ml tris buffer 1ml NADP 200µl PEP 200µl glucose 100µl ATP 100µl potassium chloride 100µl magnesium chloride 10µl hexokinase 4µl glucose 6-phosphate dehydrogenase 100µl PMS 100µl MTT	P	30min	30min

Appendix 7.1 Specific staining methods for *Wormaldia taganananа.* Modified from Richardson *et al.* (1986), Hillis and Moritz (1990) and Jackson and Resh (1991). Enzymes identified by standard abbreviations and IUBNC (1984) Enzyme Commission numbers given. Stains suspended in agarose (720mg in 50ml of water at 60°C). Bands mobile from cathode to anode, except where stated otherwise. Incubation at 37°C. No successful method was established for ACON, ADH, ALD, G6P, LAP, LDH, MPI, PEP B, PEP C, PEP D, 6PG and PYK.

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
EST Esterase E.C. 3.1.1._	2ml phosphate buffer 800µl α-naphthyl acetate 5mg fast blue RR salt	TG	20min	40min
FUM Fumarate hydratase E.C. 4.2.1.2	2ml tris buffer 1ml NAD 400µl fumaric acid 10µl malic dehydrogenase 100µl MTT 100µl PMS	CP	20min from anode	30min
αGP Glycerol 3-phosphate dehydrogenase E.C. 1.1.1.8	1ml tris buffer 1ml NADP 200µl magnesium chloride 40mg DL-α-glycerophosphate 200µl PMS 200µl MTT	TC 8.2	20min	30min
GPI Glucose 6-phosphate isomerase E.C. 5.3.1.9	1ml tris buffer 1.5ml NADP 1ml D-fructose 6-phosphate 1ml magnesium acetate 40µl glucose 6-phosphate dehydrogenase 200µl PMS 200µl MTT	TM	20min	30min
IDH Isocitrate dehydrogenase E.C. 1.1.1.42	600µl tris buffer 1.5ml NADP 1ml distilled water 500µl magnesium chloride 50mg isocitric acid 200µl PMS 200µl MTT	TC 8.2	20min	5min
MDH NAD-dependent malate dehydrogenase E.C. 1.1.1.37	400µl tris buffer 1ml NAD 700µl malic acid 200µl PMS 200µl MTT	TC 7.6	30min from centre	5min
ME	800µl tris buffer	P	40min	30min

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
NADP-dependent malate dehydrogenase E.C. 1.1.1.40	1ml NADP 1ml malic acid 100µl magnesium chloride 200µl PMS 200µl MTT			
PGM Phosphogluco-mutase 5.4.2.2	800µl tris buffer 1ml NADP 500µl magnesium acetate 400µl α-D-glucose 1-phosphate 10µl glucose 6-phosphate dehydrogenase 200µl PMS 200µl MTT	TC 7.6	20min	20min

Appendix 8.1 Specific staining methods for *Ancylus striatus*. Modified from Richardson *et al.* (1986), Hillis and Moritz (1990) and Jackson and Resh (1991). Enzymes identified by standard abbreviations and IUBNC (1984) Enzyme Commission numbers given. Stains suspended in agarose (720mg in 50ml of water at 60°C). Bands mobile from cathode to anode, except where stated otherwise. Incubation at 37°C. No successful method was established for LAP.

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
ACO Aconitase E.C. 4.2.1.3	2ml tris buffer 2ml NADP 600µl magnesium chloride 600µl aconitic acid 150µl isocitrate dehydrogenase 200µl PMS 200µl MTT	TC 7.6	20min	30min
ADH Alcohol dehydrogenase E.C. 1.1.1.1	2ml tris buffer 2ml NADP 600µl ethanol 200µl PMS 200µl MTT	TC 7.6	10min	30min
ALD Aldolase E.C. 4.1.2.13	2ml tris buffer, pH7.4 2ml NAD 2ml fructose di-phosphate 300µl arsenic acid 24µl glyceraldehyde 3-phosphate dehydrogenase 12µl triose phosphate isomerase 200µl PMS 200µl MTT	TC 7.6	20min	30min
EST Esterase E.C. 3.1.1._	2ml phosphate buffer 200µl β-naphthyl acetate 5mg fast blue RR salt	TC 7.6	20min	5min
FUM Fumarate hydratase E.C. 4.2.1.2	2ml tris buffer 2ml NAD 600µl fumaric acid 15µl malic dehydrogenase 200µl PMS 200µl MTT	TC 7.6	30min	30min
G6P Glucose 6-phosphate dehydrogenase E.C. 1.1.1.49	1ml tris buffer 1ml NADP 1ml D-glucose 6-phosphate 600µl magnesium chloride 200µl PMS 200µl MTT	TC 7.6	20min	30min
αGP Glycerol 3-phosphate dehydrogenase E.C. 1.1.1.8	1ml tris buffer 1ml NADP 400µl magnesium chloride 80mg DL-α-glycerophosphate	TC 8.2	20min	30min

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
	200µl PMS 200µl MTT			
GPI Glucose 6-phosphate isomerase E.C. 5.3.1.9	500µl tris buffer 1ml NADP 500µl D-fructose 6-phosphate 500µl magnesium acetate 5µl glucose 6-phosphate dehydrogenase 100µl PMS 100µl MTT	TC 7.6	20min	5min
IDH Isocitrate dehydrogenase E.C. 1.1.1.42	1ml tris buffer 2ml NADP 1ml distilled water 1ml magnesium chloride 100mg isocitric acid 200µl PMS 200µl MTT	TC 7.6	20min	30min
LDH L-Lactate dehydrogenase E.C. 1.1.1.27	1ml tris buffer 2ml NADP 2ml lithium lactate 200µl PMS 200µl MTT	TC7.6	20min	30min
MDH NAD-dependent malate dehydrogenase E.C. 1.1.1.37	400µl tris buffer 1ml NAD 700µl malic acid 200µl PMS 200µl MTT	TC 7.6	30min	5min
ME NADP-dependent malate dehydrogenase E.C. 1.1.1.40	2ml tris buffer 2ml NADP 2ml malic acid 300µl magnesium chloride 200µl PMS 200µl MTT	TC 7.6	40min	30min
MPI Mannose 6-phosphate isomerase E.C. 5.3.1.8	1ml tris buffer 1ml NADP 1ml D-mannose 6-phosphate 400µl magnesium chloride 28µl glucose 6-phosphate dehydrogenase 20µl phosphoglucose isomerase 200µl PMS 200µl MTT	TC 7.6	30min	30min
PEP B Leucine-glycine-glycine peptidase E.C. 3.4._._	2ml tris buffer 1ml leu-gly-gly 400µl snake venom 200µl peroxidase 200µl magnesium chloride 16mg O-dianisidine	TM	20min	1h
PEP C Glycyl L-leucine peptidase E.C. 3.4._._	2ml tris buffer 1ml gly-leu 400µl snake venom 200µl peroxidase 200µl magnesium chloride 16mg O-dianisidine	TC 7.6	30min	15min

Enzyme	Stain Composition	Run Buffer	Run Time	Inc. Time
PEP D Proline dipeptidase E.C. 3.4._._	2ml tris buffer 2ml phe-pro 1.2ml snake venom 600µl peroxidase 600µl magnesium chloride 48mg O-dianisidine	TC 7.6	20min	30min
PGM Phosphogluco- mutase E.C. 5.4.2.2	800µl tris buffer 1ml NADP 1ml magnesium acetate 800µl α-D-glucose 1-phosphate 20µl glucose 6-phosphate dehydrogenase 200µl PMS 200µl MTT	TC 7.6	20min	30min
6PG Phosphogluconate dehydrogenase E.C. 1.1.1.4	1ml tris buffer 1ml NADP 1ml 6-phosphogluconic acid 1ml magnesium chloride 200µl PMS 200µl MTT	TC 7.6	20min	15min
PYK Pyruvate kinase E.C. 2.7.1.40	2ml tris buffer 2ml NADP 600µl PEP 600µl glucose 300µl ATP 300µl potassium chloride 300µl magnesium chloride 30µl hexokinase 12µl glucose 6-phosphate dehydrogenase 200µl PMS 200µl MTT	TC 7.6	20min	5min

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